PLANT PHYSIOLOGY

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ERRATA

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Page 17, second line above table XII, for "as of" read of this.
Page 25, citation no. 22, for "Planzen gegenüberdem" read Pflanzen gegenüber dem.
Page 25, citation no. 27, for "Planzewelt" read Pflanzenwelt.
Page 46, citation no. 1, for "pur" read sur.
Page 122, citation no. 22, for "Geschwindigheit" read Geschwindigkeit.
Page 131, citation no. 12, for "Velkens van" read Welkens von.
Page 224, first line, for "Lunderardh" read Lundegardh.
Page 463, line 1, for "and" read und.
Page 445, line 14, citation no. 14, for "van" read von.
Page 464, line 5 from bottom, for "dien" read die.
Page 563, line 7 from bottom, for "determination" read determinant.
Page 585, line 22, for "Biogology" read Biology.
Page 760, line 11, for "3" read 1.

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PLANT PHYSIOLOGY

JANUARY, 1935

TOXICITY OF ALUMINUM ON SEEDLINGS AND ACTION OF CERTAIN IONS IN THE ELIMINATION OF THE TOXIC EFFECTS[1]

WALTER S. EISENMENGER

(WITH FOUR FIGURES)

Introduction

The physiological action of aluminum ions in a nutrient medium has been studied in humid areas for approximately 25 years. The relative abundance in the earth of this element would suggest frequent solution of portions, but the solution tension of aluminum compounds in nature prevents appreciable quantities from reaching the ionic stage. It has been found, however, that aluminum occurs in nearly all seed plants, which would indicate that the hydrogen ion concentration in the immediate area of roots is not the same as its concentration in the soil mass.

The toxicity of aluminum is not so easily determined as is the toxicity of the salts of the alkali and alkali earth elements. This is due to the fact that the salts of aluminum which are appreciably soluble are those of the strong acids, and these salts in turn are strongly hydrolyzed in aqueous solution, resulting in secondary formation of an acid. This in turn gives rise to hydrogen ions. The trials made may result in part from the measurement of the sum total of hydrogen and aluminum ion toxicity. This may occur in water or in soil media.

MCLEAN and GILBERT (**16**) found that the organic compound, aluminum citrate, would in part pass through a pyroxylin membrane, and that the non-diffusible colloid portion could induce toxicity.

In general the object of these experiments was to attempt to ascertain the action of aluminum on plants. It was hoped to determine: (1) the toxicity of aluminum salts of organic acids (these salts do not precipitate aluminum hydroxide when the alkalinity is raised); (2) the counter-active

[1] Contribution of the Massachusetts Agricultural Experiment Station no. 175.

effects of aluminum toxicity by the presence of a certain anion $(OH)^-$, and cations Ca^{++} and K^+.

Review of literature

The trials to determine the action of aluminum on plants in nutrient solutions and in soil have been numerous. The harmful effects of the aluminum ion in water are not difficult to demonstrate, but when attempts are made with a three phase and heterogeneous medium such as soil, where the varied hydrogen ion concentration, adsorptive properties, varied quantities if water, and diverse activity of microorganisms enter the picture, the matter is largely one of circumstantial evidence with respect to evaluating the deleterious effects of aluminum.

The reaction of plants to aluminum is in a measure dependent upon their degree of adaptation to their habitat. Thus plants which are closely related to those of the prehistoric past subsist on less potassium, calcium, and magnesium and have in their ash abundant quantities of aluminum, iron, and silica. STOKLASA (**27**) has found that xerophytes and mesophytes are sensitive to aluminum while hydrophytes are to a greater degree resistant to the element. One of the lycopods contains slightly more than 35 per cent. of aluminum oxide in its ash, while a plant like the common pea may contain but 20 p.p.m. in the ash (**2**). Mesophytes that by chance grow in a medium of moist or marshy ground contain more aluminum than those adapted to cultivated soil.

The harmful effects of aluminum as suggested by STOKLASA (**29**) are contractions of the protoplasm, and stronger solutions may soften or even dissolve this substance. He found that when strong harmful concentrations were used there was less aluminum absorbed by the plant than when more dilute solutions were used, and from this concludes that the process is not one of simple absorption but one of alteration of permeability and disturbance of the cell colloids. This eventually leads to outward passage of calcium, potassium, and magnesium. The aluminum in turn prevents entrance of excess iron into the tissues. SKEEN (**25**) has found that calcium ions have an antagonistic effect upon the aluminum which causes the toxicity. Also MCLEAN and GILBERT (**15**) have demonstrated that aluminum poisoning was accompanied by reduced absorption of dyes, of nitrates, and of water. The aluminum was found for the most part in the cortex and concentrated in the nucleus of the protoplasm.

RUPRECHT (**23**) states that the first layer or two of cells in the growing part seem to be principally affected. The harm was probably due finally to lack of nourishment rather than to poisoning itself. SZÜCS (**31**) concludes that aluminum, like yttrium and lanthanum, has the capacity to retard plasmolysis instead of increasing permeability as was formerly thought. This

effect is to retard the relative capacity to take up other nutrients. It solidifies protoplasm. DENISON (**7**) found that aluminum salts stimulate ammonifying organisms but act adversely upon nitrifying bacteria. MCGEORGE (**13**) finds that sugar cane grown in acid soils containing soluble iron and aluminum is characterized by abnormal accumulations of these elements in the nuclei surrounding the xylem cells at the nodal point of the stalk. MAGISTAD (**17**) states that water cultures of aluminum were found to prevent the formation of lateral rootlets on barley, but not on rye.

INCREASED GROWTH RESPONSE DUE TO ALUMINUM.—ROTHERT (**22**), one of the first investigators of aluminum toxicity, reported that definite limited amounts of aluminum salts may stimulate plant development. MCLEAN and GILBERT (**16**) found that concentrations of aluminum in solutions of low concentration, such as 3–13 p.p.m., stimulate growth. SOMMER (**26**), by careful purification of salts, found that millet showed a markedly increased growth rate due to the presence of aluminum in the solution. The effect was especially marked with respect to the quality of the seeds. MAZÉ (**18**), also by using extreme care in purification of salts, found aluminum essential for adequate development of corn. STOKLASA (**28**), by using a low concentration (0.0002 atomic weight of aluminum per liter) obtained increased growth of plants as compared with those solutions containing no aluminum.

In the plant world there is obviously an extreme variance of tolerance and requirement as concerns elements; and a generalization or incompatible comparison is always misleading. The indirect effects of aluminum may not be due to the aluminum but to the acidity produced by the salt. This is especially true for the aluminum salts of strong acids.

COVILLE (**6**), in order to attain a high acidity for acid loving plants, *Rhododendron, Vaccinium, Franklina,* and *Hydrangea,* added aluminum sulphate and obtained remarkable results in soils that had become too alkaline.

CONDITIONS CONDUCIVE TO ACTIVE ALUMINUM.—Active aluminum is found more abundantly (1) in acid soils, (2) in soils low in organic matter, (3) in humid areas when the seasonal water supplies are low, and (4) in arid regions when the alkalinity becomes high. STOKLASA (**30**) states, "the richer the soil in decomposed organic matter the stronger the combination of aluminum sulphate and aluminum chloride which can be used without injury to plant life." GILBERT and PEMBER (**9**) found the dry weight yields of barley plants grown in samples of acid soils from several soil types, widely separated geographically, very closely correlated with active aluminum. This corresponds with the findings of PIERRE, POHLMAN, GORDON, and MCILVAINE (**21**). TURNER (**34**) found that aluminum is invariably actively present only in soils with pH values less than 5.1. The amount of replaceable aluminum, however, is not regular with respect to hydrogen ion con-

centration. MIYAKE (**20**) reports that the toxicity of aluminum chloride for rice seems to be approximately equal to that of hydrochloric acid of the same normality, but that the concentration of hydrogen ions found formed by the hydrolysis of aluminum chloride is less than that formed by dissociation of hydrochloric acid of the same normality.

It is stated by MAGISTAD (**17**) that data relating to the solubility of aluminum in water and soil solutions show that at pH 5 there are 1–2 p.p.m. of aluminum oxide equivalent in solution. As the acidity decreases to the neutral point the solubility decreases almost to zero. When the acidity becomes greater than pH 5 the solubility increases until pH 4.5 is reached, at which point the solubility increases rapidly. Strongly alkaline soils were found to contain much aluminum. One, at pH 9.01, contained 31 p.p.m. These data are analogous to those of LIGON and PIERRE (**11**).

MCGEORGE (**13**) states that acidity *per se* or hydrogen ion concentration of the intensity present in our most acid soils has absolutely no influence upon growth of sugar cane. It is, he states, the aluminum salts which are present in such soil types that retard plant growth. MCGEORGE, BREAZEALE, and BURGESS (**14**), in their work on electrometric titration measurements of the solubility of aluminum compounds at different hydrogen ion concentrations, conclude that from pH 5 to 7.5 aluminum is almost completely insoluble, while at pH 8 the aluminate forms. Black alkali soils contain appreciable amounts of water soluble aluminates.

TOLERANCE OF DIFFERENT PLANTS.—MCLEAN and GILBERT (**15**) found considerable variance in plant tolerance. Most sensitive are lettuce, beets, timothy, and barley; medium sensitive are sorghum, cabbage, oats, and rye; resistant are corn, turnips, and red top (the last is especially resistant). SKEEN (**25**) found the white lupine to be about three times as resistant to aluminum as is the kidney bean.

REMEDIAL MEASURES FOR ALUMINUM TOXICITY.—The suggestions that have been made for alleviating the toxicity of aluminum have been the application of neutralizing agents and buffers. Also the calcium ion has been demonstrated to overcome it (**25**). It has been found that lime, phosphate, and organic matter remedy the bad effects of aluminum. In reclamation of alkaline lands summer leaching has been suggested for elimination of soluble aluminum compounds.

Other workers have made valuable suggestions as to active aluminum occurrence and agencies for its elimination (**5, 10, 19, 7, 20, 3, 4, 1, 24**).

OPPONENTS OF THEORY OF ALUMINUM TOXICITY IN SOILS.—Owing to the apparent action of aluminum in acid solutions and soils, the question has been raised as to which ion, the H^+ or Al^{+++}, is the one that is destructive to plant tissue. Thus after years of experimentation, adversaries of the aluminum toxicity theory maintain that inaccurate observations account for

the appraisal of this element in toxicity. STOKLASA (**30**) says: "The statement that aluminum compounds in the soil have an injurious effect on plant growth is based on no exact experimental foundation." LINE (**12**) states regarding the toxic aluminum theory of soils that "old evidence and fresh experimental work seem to show that this theory is no longer tenable," that most of the aluminum in the water is in the form of colloidal hydroxide.

Methods

Aluminum citrate and aluminum tartrate were the salts used. Seedling stages of the soy bean (variety Manchu), corn (variety Sweepstakes), and Japanese buckwheat were the plants employed in the growth trials.

The seeds were germinated in a dish in the dark, and when the sprouts were about 10 mm. long they were transferred to the growth medium. The sprouts of the buckwheat were about 8 mm. long. The roots dipped into the solution.

CRITERIA OF GROWTH.—Three criteria of growth were used: (1) root elongation, (2) length of top, and (3) total dry weight. There is greater simplicity with respect to behavior in the seedling stage than in the later periods of growth. In the early stages of growth the plant is well supplied by ions, and the organic matter of the seed is sufficient to afford protection from starvation of plants grown in distilled water. The protoplasm seems to be reactive to the toxic constituents of the solution.

The investigation involved the toxicity of aluminum citrate for corn; aluminum citrate and tartrate for soy beans; and aluminum tartrate for buckwheat. The concentration of each salt ranged from 0.00012 to 0.006 M, or 2, 5, 15, 30, 50, 70, 85, 95, 98, and 100 per cent. of 0.006 M. Tests were also made to show the effects upon growth of mixtures of the aluminum salt and calcium nitrate, calcium hydroxide (freshly prepared), and potassium hydroxide. In the case of the mixtures eleven sets of percentage molecular proportions were used: $0+100$, $2+98$, $5+95$, $15+85$, $30+70$, $50+50$, $70+30$, $85+15$, $95+5$, $98+2$, and $100+0$.

The methods relating to roots were essentially the same as those described by TRELEASE and TRELEASE (**32**) and by EISENMENGER (**8**). For each culture two pyrex beakers (tall form without lip) were used. The smaller beaker was of 300-cc. capacity, the larger of 600-cc. capacity. Over the top of the small beaker was stretched a piece of paraffined mosquito netting which was secured below the rim by a ligature of paraffined thread. The smaller beaker was placed inside the larger one, and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the seedlings from the germinating dish were of the required length they were placed on the mosquito netting so that the roots dipped

into the culture solution. Duplicate cultures of 25 plants were used for each experimental solution, a total of 50 plants for each concentration. The cultures were placed in a dark room and the seedlings were allowed to grow until the primary roots of the control culture had acquired an average length of about 95 mm., or until these roots had elongated 85 and 87 mm. (95–10 or 95–8).

The length of the primary root, the maximum length of top, and the total dry weight of each individual plant were then recorded and the average computed. From the average of root length was deducted the average length of the roots at the time the seedlings were taken from the germinating dish. The difference constituted the average root elongation value for the culture. The growth data here presented are relative values. Each relative growth value was obtained by dividing the average elongation of root, top, and total dry weight of a given culture by the average elongation of root, top, and total dry weight of the control culture and multiplying this quotient by 100. With each group were four beakers containing 25 plants each in a complete nutrient medium. These 200 plants were designated as controls. For corn the control was Knop's solution. For soy beans and buckwheat the control contained: $CaHPO_4$, 0.0011 M; $MgSO_4$, 0.0057 M; $CaSO_4$, 0.0013 M; KCl, 0.0037 M; and KNO_3, 0.007 M. There was little difference in the controls with respect to reactivity toward plants, but for soy beans the latter was slightly more favorable than was Knop's.

The temperature during the growth period of the seedlings varied from 21° to 22° C. The time required for the roots of the control solution to acquire an additional length of 85 or 87 mm. varied from 95 hours for buckwheat to 116 hours for corn. When a series of single salt solutions was used, two additional cultures of 25 plants each grown in distilled water were used. The distilled water was obtained from a Barnstead still.

Discussion

Corn

Roots.—As previously stated, corn has been regarded as one of the plants more resistant to aluminum toxicity. In the present experiment, however, 15 per cent. of 0.006 M aluminum citrate exerted a decidedly retarding effect on the growth of corn roots. This amount is approximately equal to 22 p.p.m. of aluminum (fig. 1 *B*). Amounts higher than this exerted only a slightly increased toxicity on roots compared with that at 15 per cent. of 0.006 M. The plants thus affected showed lesions of the primary root principally. The end of this root eventually assumed the nature of an open wound. The lateral roots showed no such effects except those branching from near the injury. (See table I for comparative data.)

TABLE I

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	REACTION
%	%	%	%	*pH*
66.3	53.8	68.4	0	5.82
83.5	62.1	88.0	2	4.75
48.6	51.1	76.6	5	4.50
24.8	50.9	67.7	15	4.50
18.7	32.5	52.1	30	4.50
12.6	32.2	47.1	50	4.05
16.8	29.4	47.5	70	3.64
11.4	29.4	39.0	85	3.55
11.9	22.4	39.5	95	3.55
13.3	24.9	42.5	98	3.50
7.2	13.4	34.9	100	3.43

Roots are by far the most injured by the toxic solution; their variation due to variable concentration causes more marked breaks in the growth curve than is the case with curves of the relative length of tops and curves of the total dry weight. Thus the relative length of tops and relative total dry weight (fig. 1 *B, C*) indicate that the greatest deviation occurs between concentrations of 15 and 30 per cent. of 0.006 M, while the greatest deviation in relative root length occurs between 5 and 15 per cent. of 0.006 M.

TOPS AND TOTAL DRY WEIGHT.—The relative diminution due to aluminum citrate is greater for the tops than for total dry weight. In view of the fact that the diminution of relative length of roots is most pronounced,

TABLE II

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	*pH*
66.4	53.9	68.8	0	6.61
93.1	55.2	75.2	2	7.85
117.1	68.4	87.5	5	7.85
121.6	74.3	97.9	15	9.0
111.7	80.2	82.6	30	9.6
80.5	63.6	88.0	50	9.6
57.7	53.9	79.5	70	9.6
32.0	28.9	62.3	85	9.6
25.0	30.6	71.1	95	9.6
26.1	37.6	69.5	98	A*
26.9	44.9	71.2	100	A*

* A indicates values greater than preceding figure.

TABLE III

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
27.0	44.9	71.2	0	100	V*
23.0	50.0	77.0	2	98	9.0
16.8	46.6	82.0	5	95	9.0
41.7	66.5	113.4	15	85	8.8
84.5	69.2	100.7	30	70	8.37
126.7	78.3	110.6	50	50	5.60
94.5	57.2	78.9	70	30	4.18
42.2	36.1	62.5	85	15	3.70
14.0	20.6	49.1	95	5	3.40
12.4	11.3	42.5	98	2	3.40
7.2	13.4	34.6	100	0	3.43

* V indicates values above pH 9.0.

it is probable that the plants affected contained less water or had more thickened anatomical parts than the normal plants, as is the case in some pathological conditions. The corn grown in the higher aluminum concentrations probably was competing more for water than were normal plants.

MIXTURES OF ALUMINUM CITRATE AND CALCIUM HYDROXIDE.—In growing plants in mixtures of the salts of the more electro-positive elements, the relative effects are more easily shown, for the growth effects are for the most part those produced by the cations. When mixtures containing aluminum salts are concerned a more complex relationship is introduced, owing to the secondary products of hydrolysis. Thus in the solutions of all aluminum salts

TABLE IV

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	*pH*
66.4	53.8	68.8	0	6.61
96.1	82.1	89.5	2	6.67
94.1	82.1	95.9	5	6.37
99.9	97.0	97.8	15	6.30
92.3	97.8	97.4	30	6.25
78.9	85.8	76.7	50	6.25
83.0	90.7	83.3	70	6.14
87.8	93.1	91.9	85	5.95
81.1	84.3	88.6	95	5.95
78.0	93.4	83.6	98	5.95
75.5	78.1	77.0	100	5.51

TABLE V

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	%	*pH*
75.5	78.1	77.0	0	100	5.07
88.7	48.2	71.3	2	98	4.33
77.1	76.1	70.8	5	95	4.05
55.7	65.7	62.8	15	85	3.85
62.4	63.9	70.5	30	70	3.68
45.9	46.6	54.0	50	50	3.66
32.2	41.6	57.9	70	30	3.50
20.2	35.2	57.5	85	15	3.50
10.3	17.7	40.8	95	5	3.50
3.6	12.4	30.3	98	2	3.50
7.2	13.4	34.9	100	0	3.51

the cation gives rise to hydrogen ions in measurable quantities. We are not dealing with two elements, which are cations, but in addition the cation hydrogen ions in excess of hydroxyl anions. The influence of the additional ions renders interpretation of relative growth data more difficult. In this investigation growth data were compared when corn seedlings were grown in aluminum citrate alone, in calcium hydroxide, and in mixtures of the two compounds. The total molecular concentration was not changed, 0.006 M of calcium hydroxide and aluminum citrate.

In order to express more adequately the effects of these compounds when

TABLE VI

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + POTASSIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M POTASSIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
13.6	52.9	79.8	0	100	9.8
31.7	52.3	77.8	2	98	9.7
16.7	36.1	65.8	5	95	9.7
18.5	38.4	74.5	15	85	9.6
25.2	49.0	79.1	30	70	9.0
73.8	69.6	94.3	50	50	8.36
32.0	43.5	68.0	70	30	6.00
14.3	36.5	70.4	85	15	4.15
11.9	29.2	67.1	95	5	3.84
12.1	25.6	64.1	98	2	3.45
7.2	13.4	34.9	100	0	3.43

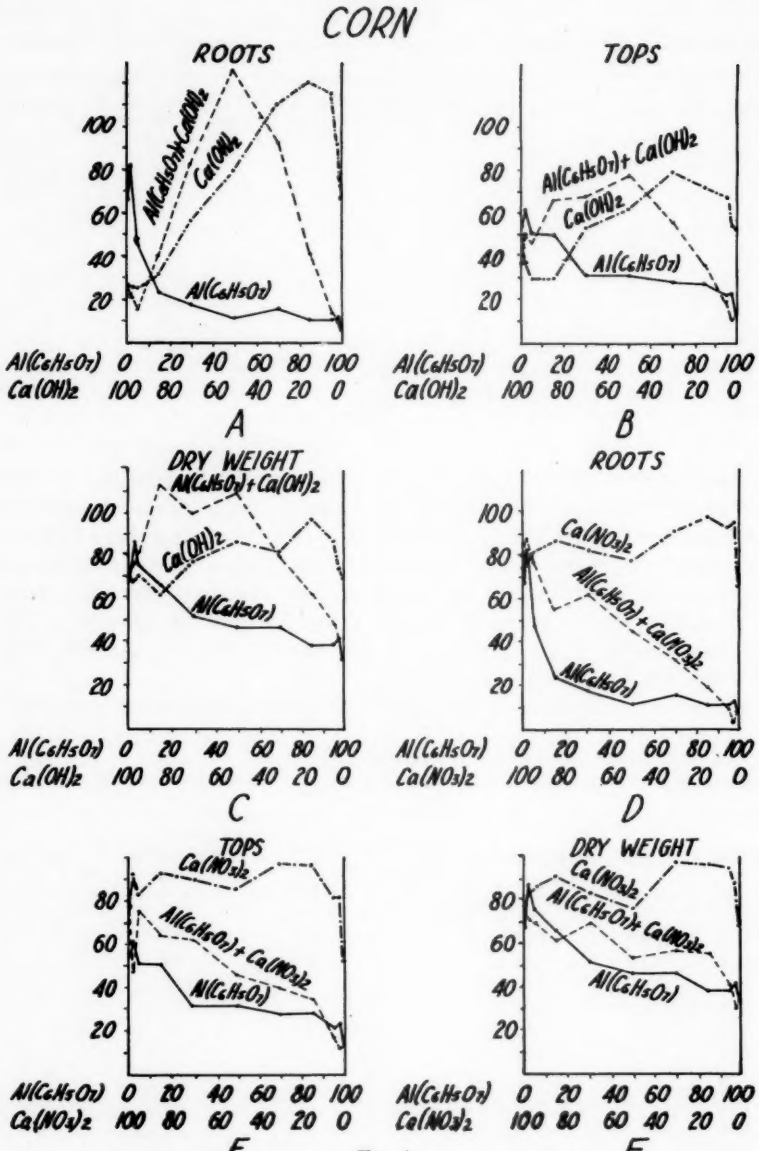

FIG. 1

A, B, C, D, E, F: Elongation of corn roots and tops together with total dry weight in simple solutions and mixtures of aluminum citrate and calcium hydroxide, also in simple solutions and mixtures of aluminum citrate and calcium nitrate. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

in mixture, it is essential to know what is the effect of calcium hydroxide when used singly (see table II). The molarity was based on 0.006 M calcium hydroxide. The curve (fig. 1 *A*) would indicate that low concentrations of calcium hydroxide are stimulating to growth but that at higher concentrations the relative growth curve rapidly falls for the relative root length, and to a lesser degree for tops and total dry weight. The deleterious effect is not to a great extent due to the calcium ion, but rather to the hydroxyl ion. For all cases the range of toxicity is greater for the aluminum salt than for the calcium base (fig. 1 *A, B, C*).

One of the most outstanding characteristics of growth relationships is the abnormal stimulation to growth of roots in the seedling stage when the plants are grown in mixtures of aluminum citrate and calcium hydroxide (table III). In part this is due to the neutralization of the acidic properties of the aluminum compounds. The neutralization is not the cause of the increased growth, however, for roots and total dry weight are greater than the growth results with either the aluminum salt or the calcium base. This reaction does not confine itself to single groups, but applies to all plants used in the experiment, which represent the mean of thousands of plants. It would seem that a neutral salt would indicate the effect of calcium ion without the accompanying neutralization of the hydroxyl ion.

It may be stated that the combined action of the hydroxyl ion and the calcium ion results in a marked effect in overcoming the toxicity of the aluminum and the hydrogen ions in growth trials.

GROWTH RESULTS OF MIXTURES OF CALCIUM NITRATE AND ALUMINUM CITRATE.—In single salt solutions one of the salts least toxic to seedlings is calcium nitrate in dilute solutions (**8**). The low toxicity makes it difficult to secure consistent growth results (table IV). The growth of corn seedlings in varying percentage proportions of 0.006 M was not greatly reduced from that of the controls. It does not modify to any degree the hydrogen ion concentration of a medium in which it is placed. When various molecular proportions of calcium nitrate and aluminum citrate are mixed, the results indicate that the aluminum ions and hydrogen ions increase the toxicity of the solution as compared with the results obtained from the calcium nitrate salt used singly, but that the calcium salt ameliorates the toxic effects of the aluminum ion. It can definitely be stated that the calcium ion exerts an antagonistic action on the aluminum ion as concerns growth promotion. As is shown by the relative growth promoted by the addition of calcium and hydroxyl ion formed, however, the nearly neutral salt in no instance has the extreme action that is seen when calcium oxide is used instead of the nitrate (fig. 1 *D, F* and table V).

ALUMINUM CITRATE AND POTASSIUM HYDROXIDE.—Potassium hydroxide was used in mixtures with aluminum citrate to determine to what extent it

TABLE VII

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF CITRIC ACID EQUIVALENT IN ACIDITY TO CORRESPONDING ALUMINUM SALT

ROOTS	TOPS	DRY WEIGHT	EQUIVALENT IN pH OF ALUMINUM CITRATE PERCENTAGE 0.006 M	REACTION
%	%	%	*pH*	*pH*
66.4	53.9	68.8	0	5.82
72.3	68.6	77.8	2	4.75
89.0	62.4	65.4	5	4.50
72.2	75.6	83.3	15	4.50
70.1	81.6	93.1	30	4.50
51.4	66.5	78.0	50	4.05
34.1	39.9	70.7	70	3.64
25.0	45.5	57.3	85	3.55
4.5	25.2	45.4	95	3.55
15.7	42.2	60.9	98	3.50
10.2	41.7	54.8	100	3.43

would resemble calcium hydroxide in growth promoting properties when mixed with the same aluminum salt. In no instance did it cause the abnormally large growth which occurred with the calcium base. There was low toxicity over a wide range of concentration of the mixture as shown by total dry weight. For roots and tops a point was attained where marked improvement occurred, 50 per cent. of 0.006 M of each component. On one side of this point the hydrogen and aluminum ions were apparently too

TABLE VIII

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + POTASSIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M POTASSIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
16.9	38.1	49.4	0	100	9.8
8.6	34.7	47.8	2	98	9.4
18.2	38.5	50.8	5	95	9.2
10.1	41.4	65.8	15	85	9.0
67.8	64.4	62.4	30	70	9.0
14.2	28.9	47.2	50	50	7.66
6.0	17.8	36.9	70	30	5.30
3.0	13.9	36.4	85	15	4.15
4.3	11.0	29.3	95	5	3.85
1.4	11.8	26.2	98	2	3.56
4.8	18.0	31.4	100	0	3.45

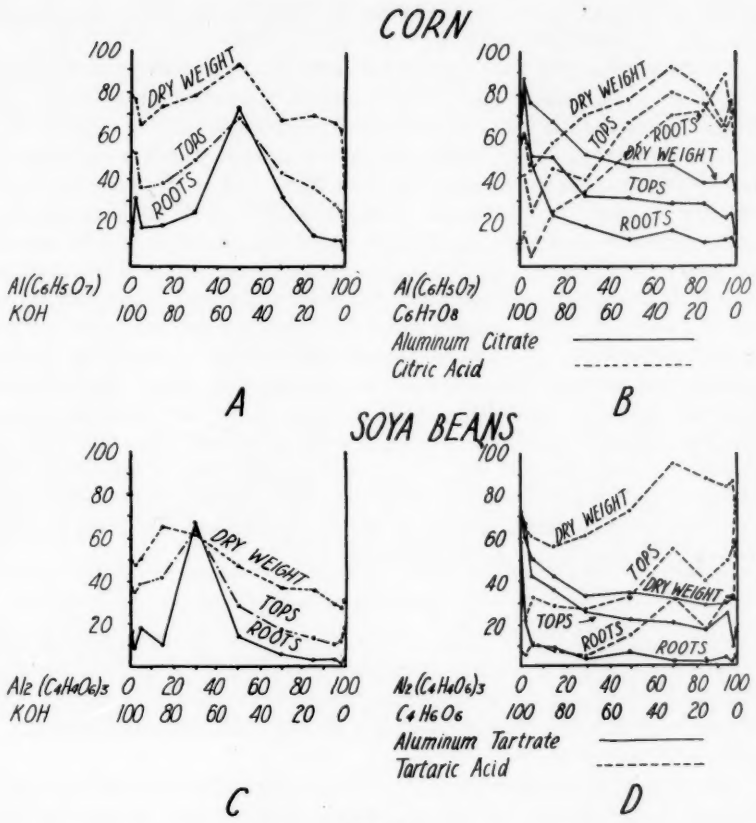

FIG. 2

A: Elongation of corn roots and tops together with total dry weight of corn seedlings grown in simple and mixed solutions of aluminum citrate and potassium hydroxide.

B: Elongation of corn roots and tops in addition to total dry weight of seedlings grown in aluminum citrate, and in citric acid, the latter of sufficient concentration to make the acid solution of pH equal to the corresponding aluminum salt solutions.

C: Elongation of soy bean roots and tops in addition to dry weight of seedlings grown in simple and mixed solutions of aluminum tartrate and potassium hydroxide.

D: Elongation of soy bean roots and tops in addition to total dry weight of seedlings grown in aluminum tartrate and tartaric acid. Concentration of the acid was sufficient to make it of pH equal to the corresponding salt solutions.

active, with little antagonism of the potassium ion toward the aluminum; and on the other side the alkalinity was too pronounced. The general outline of the curve would suggest a point of neutralization for best relative

growth with meager action of potassium in retarding the influence of aluminum (fig. 2 *A* and table VI).

COMPARATIVE EFFECT OF ALUMINUM CITRATE AND CORRESPONDING CITRIC ACID.—Some controversy has arisen regarding the extent to which the hydrogen ion causes the deleterious effects in aluminum toxicity experiments. In order to make an approximate comparison, corn seedlings were grown in water solutions that were made acid with citric acid to the same extent as were the corresponding aluminum citrate solutions. This was done by comparing indicator reactions. In the extremes of low concentration the growth was not very different. As the concentration of the salt and the acid increased there was a gradual fall in the relative growth. The fall in the growth curve (fig. 2 *B*) is more rapid for the aluminum salt than for the corresponding acid. Thus at 50 per cent. of 0.006 M, the relative growth value of corn roots for aluminum salt is approximately 12 as compared with 51 for the acid (see table VII). At 30 per cent. of the same total concentration for the total aluminum salt concentration on corn roots the value is about 18, and for the acid, 70. This would suggest that the toxicity of aluminum is always augmented by hydrogen ions, but the relative effects are not nearly of equal value. It may be stated that much of the actual toxicity of aluminum salts is due to the aluminum ion.

SOY BEANS

Soy beans were grown during the seedling stage in various percentage proportions of 0.006 M aluminum tartrate and citrate. Aluminum tartrate contains proportionately twice as much aluminum as does the same molarity of aluminum citrate. Tartaric acid is a dibasic acid, while the citrate is a salt of a tribasic acid.

ROOTS.—At a concentration of 5 per cent. of 0.006 M aluminum tartrate, the toxicity toward the root system is nearly equal to the maximum, equivalent to about 16 p.p.m. of aluminum.

TOPS AND TOTAL DRY WEIGHT.—The maximum toxicity as indicated by tops and total dry weight is not attained until the plants are grown in a concentration equal to 30 per cent. of 0.006 M. This is only slightly less than 100 p.p.m. (fig. 3 *A, B, C*). At this concentration and higher the roots seemingly did not grow at all.

As with corn, the soy beans were grown in solutions containing fractional parts of the total molecular concentration, 0.006 M calcium hydroxide. At moderately low concentrations there is a marked stimulation to growth in the seedling stage. This increase is measurably greater than that of the control. Although soy beans have been classified as of low lime requirement (**33**), concentrations of calcium hydroxide equivalent to 0.0018 M of calcium appear to augment growth above the control for roots of the beans.

TABLE IX

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF TARTARIC ACID

ROOTS	TOPS	DRY WEIGHT	TARTARIC ACID EQUIVALENT TO pH OF CORRESPONDING PERCENTAGE OF ALUMINUM SALT	REACTION
%	%	%	%	*pH*
59.6	73.3	76.8	0	6.61
32.8	55.5	87.4	2	4.45
32.4	49.4	84.5	5	4.44
17.8	40.1	88.9	15	4.25
32.1	55.7	95.8	30	3.80
15.5	33.0	73.2	50	3.62
5.9	27.5	61.4	70	3.54
8.3	29.7	56.2	85	3.54
11.9	33.6	61.3	95	3.45
6.9	24.6	65.8	98	3.49
7.5	38.8	66.8	100	3.45

Above this concentration the growth of roots falls rapidly. The various concentrations of calcium oxide do not materially affect the relative growth of tops and total dry weight. The lowest concentration is not appreciably different in affecting growth promotion from that of the highest concentration.

MIXTURES OF ALUMINUM TARTRATE AND CALCIUM HYDROXIDE.—When the aluminum salt and the calcium base are mixed in various proportions the growth stimulation properties are changed. The marked relative growth is indicative when the proportions of 0.006 M are 30 per cent. of the aluminum salt and 70 per cent. of the base. The pH of the solution promoting maximum

TABLE X

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	REACTION
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
21.4	66.7	61.6	2	4.75
10.9	42.5	50.9	5	4.44
9.8	36.8	52.9	15	4.25
4.5	26.9	33.2	30	3.80
6.6	22.5	34.8	50	3.62
0.4	20.9	32.2	70	3.54
0.5	17.5	29.0	85	3.54
3.0	25.0	29.5	95	3.45
0.5	9.9	31.9	98	3.45
4.8	18.0	31.4	100	3.45

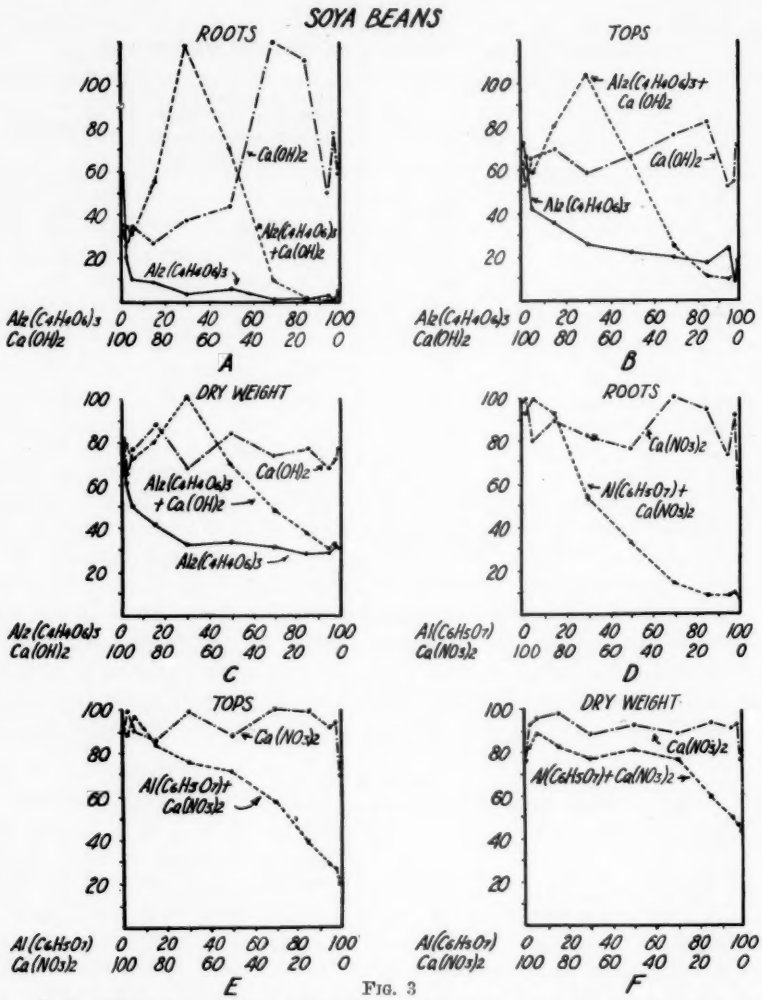

FIG. 3

A, B: Elongation of roots and tops of soy beans grown in simple and mixed solutions of aluminum tartrate and calcium hydroxide. Ordinates represent percentages of elongation for standard solution; abscissas represent percentage molecular proportions of 0.006 M.

C: Dry weight of soy beans grown in simple solutions and mixture of aluminum tartrate and calcium hydroxide.

D: Elongation of roots of soy beans grown in simple salt solution and mixtures of aluminum citrate and calcium nitrate.

E: Elongation of tops of soy beans grown in single salt solutions and in mixtures of aluminum tartrate and calcium nitrate.

F: Dry weight of soy beans grown in single salt solutions and mixtures of aluminum tartrate and calcium nitrate. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

TABLE XI

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
78.7	55.5	73.0	2	7.85
50.6	53.2	67.6	5	7.85
111.2	83.0	77.3	15	8.10
120.0	76.9	74.6	30	9.7
44.2	66.8	84.3	50	9.7
38.3	59.1	67.5	70	9.7
27.6	70.1	88.7	85	9.8
35.1	66.4	76.8	95	9.8
24.2	53.5	64.6	98	A
36.5	64.7	82.5	100	A

growth in single calcium hydroxide solutions was slightly above 9 (table XI). The pH of the mixture solution yielding best relative growth was about 6.5 (table XII). The beneficial effects of the mixture were in part due to calcium ion with an accompanying optimum pH for this particular concentration. The abrupt drop in growth curve suggests neutralization. It might be added that calcium hydroxide and aluminum salts are the only mixtures used that yield results in which the stimulation to growth in some instances is higher than that of the controls. The writer has not a positive explanation as of behavior. The comparison is made with mixtures of aluminum salt and cal-

TABLE XII

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
36.6	64.7	82.5	0	100	.....
35.3	62.2	79.9	2	98	.....
31.4	59.4	72.8	5	95	.....
54.1	81.1	80.1	15	85	7.65
118.1	104.3	100.8	30	70	6.50
71.5	65.4	70.3	50	50	5.66
10.3	25.4	48.9	70	30	4.18
1.2	11.8	38.6	85	15	3.70
0.0	10.0	31.0	95	5	3.60
1.3	12.0	33.5	98	2	3.60
4.8	18.0	31.3	100	0	3.45

TABLE XIII

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
92.5	94.8	92.8	2	6.65
74.0	92.5	91.0	5	6.37
95.0	99.8	93.4	15	6.30
100.2	100.7	89.0	30	6.25
76.7	88.4	92.7	50	6.14
82.8	99.4	88.3	70	5.95
89.4	85.3	97.0	85	5.95
80.1	90.1	95.9	95	5.95
99.3	99.5	92.9	98	5.95
98.6	90.7	76.0	100	5.51

cium nitrate, and mixtures of aluminum salt and potassium hydroxide. This would suggest that an expression of pH is more significant when stated in relationship to other elements than as an entity itself.

MIXTURES OF ALUMINUM CITRATE AND CALCIUM NITRATE.—The toxicity of calcium nitrate toward soy bean seedlings is only slight, approximately like that of the same salt toward corn (table XIII).

Mixtures of calcium nitrate and aluminum citrate are more indicative of an approximate mean of the toxicity of calcium nitrate and aluminum citrate each used singly. Another trial of a series of concentrations with

TABLE XIV

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	%	*pH*
93.0	90.7	81.2	0	100	5.07
92.8	87.9	81.9	2	98	4.33
99.6	96.4	88.8	5	95	4.05
92.3	84.0	82.6	15	85	3.85
54.0	75.8	77.3	30	70	3.68
33.2	71.9	81.0	50	50	3.66
14.8	57.8	76.7	70	30	3.50
8.7	39.3	59.5	85	15	3.50
8.6	28.6	50.1	95	5	3.50
9.1	26.9	46.8	98	2	3.50
8.1	19.7	43.8	100	0	3.51

TABLE XV

GROWTH OF BUCKWHEAT SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	REACTION
%	%	%	%	*pH*
5.7	13.5	29.7	0	6.61
9.0	13.5	25.1	2	4.75
3.7	11.3	29.9	5	4.44
3.1	10.1	33.7	15	4.25
15.1	15.2	56.3	30	3.80
13.4	12.0	61.3	50	3.62
14.8	14.3	53.7	70	3.54
12.1	10.4	52.3	85	3.54
14.3	10.7	31.6	95	3.45
5.3	9.4	39.4	98	3.49
11.5	8.1	20.0	100	3.45

aluminum citrate used singly would have lent itself to a fuller explanation of this action. The two growth curves, however, that of calcium nitrate and that of aluminum citrate and calcium nitrate, are not greatly different from those when corn was used; it thus seems a reasonable conclusion that the growth curve of the single aluminum salt would not be greatly different from that for corn (table XIV).

The calcium ion effect on total dry weight suggests a definite detoxifying effect. As in corn, this is more pronounced for relative total dry weight and relative length of top than for roots (fig. 3 *D, E, F*).

TABLE XVI

GROWTH OF BUCKWHEAT SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
127.9	70.9	93.7	0	100	V
177.5	73.6	94.0	2	98	9.0
194.7	66.6	86.6	5	95	8.0
168.3	66.4	77.4	15	85	7.65
81.0	52.8	90.1	30	70	6.50
21.9	17.9	43.2	50	50	5.60
22.2	10.2	24.3	70	30	4.18
16.7	11.4	21.1	85	15	3.70
17.7	8.1	16.1	95	5	3.40
16.2	7.4	20.5	98	2	3.40
11.5	8.1	20.0	100	0	3.40

MIXTURES OF ALUMINUM TARTRATE AND POTASSIUM HYDROXIDE.—To determine the effect of the different hydrogen ion concentrations, bean plants were grown in mixtures of the two compounds aluminum tartrate and potassium hydroxide. As with corn, there seemed to be a rather definite point for favorable growth promotion. The potassium ion here as before did not seem to overcome the toxicity to any great extent; but at one concentration, 30 per cent. aluminum tartrate, 0.006 M, and 70 per cent. potassium hydroxide, 0.006 M, there was a fair medium for growth promotion. It is the writer's opinion that it is more of an index of favorable hydrogen ion concentration than a favorable mixture of the other cations, aluminum and potassium. The pH for the best growth in this trial was between 8 and 9 (table VIII). This was approximately the same as for corn in the aluminum citrate and potassium hydroxide mixture.

TABLE XVII

GROWTH OF BUCKWHEAT SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + POTASSIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	PERCENTAGE 0.006 M POTASSIUM HYDROXIDE	REACTION
%	%	%	%	%	*pH*
1.6	9.2	24.9	0	100	9.6
2.8	9.4	28.4	2	98	9.4
3.1	10.3	26.7	5	95	9.2
16.4	13.8	31.2	15	85	9.0
87.6	78.7	71.5	30	70	9.0
13.7	37.2	44.4	50	50	7.66
4.3	13.7	24.6	70	30	5.30
4.0	10.1	23.5	85	15	4.15
3.1	6.5	12.0	95	5	3.84
3.5	6.5	10.7	98	2	3.56
11.7	8.1	20.0	100	0	3.45

It is worthy of note that the seemingly optimum pH of solutions for the seedling stage is not like that of most soils for yielding crops. The optima are higher for solutions. For permanent growth in soils at high pH much of the common vegetation of a humid region would not thrive so well as at a lower pH. This may be due to precipitation and complete isolation of some required element of the field, or it may be possible that the hydroxyl ion only slowly permeates the protoplasm of the seed and does not disturb the equilibrium during the short time the seedling gets its nutrients from the seed.

COMPARISON OF TOXICITY OF ALUMINUM TARTRATE AND TARTARIC ACID.—Water solutions were made acid with tartaric acid, comparable to the acidity

of the corresponding aluminum salt, aluminum tartrate, at different concentrations and soy bean seedlings were grown in the solution. This was a similar procedure to that adopted for corn. The results are as with corn in citric acid. The aluminum salt solutions with hydrogen ion concentrations the same as those of the corresponding acid caused lower relative growth rates than did the acid. This was the case for roots, tops, and total dry weight (fig. 2 *D* and tables IX and X).

Buckwheat

Corn and soy beans bore considerable resemblance in their reaction to aluminum salts. Buckwheat in certain respects demonstrates a different behavior. This plant in the seedling stage is seemingly more sensitive to distilled water-low osmotic pressures. This was indicated by the results of several trials, each trial including 50 seedlings. Also for single salt cultures of aluminum tartrate there is little difference between the comparative lengths of tops and roots (table XV). The plant seems to be more sensitive to low concentrations than it is to moderate concentrations of aluminum tartrate. At 0.0018–0.003 M the relative growth is better for roots, tops, and total dry weight than it is at lower and higher concentrations of the same salt. In other experiments the relative root length was the most pronounced with respect to retardation of relative growth. In the case of buckwheat the relative top length is almost equally affected. The total dry weight is higher, suggesting low water content or thickening and branching of stems and roots.

Mixtures of aluminum tartrate and calcium hydroxide.—Relative growth of buckwheat is promoted by the higher concentrations of lime rather than by the lower concentrations. The root system is most markedly affected by high proportions of calcium hydroxide (approximately 95 per cent. 0.006 M calcium oxide and 5 per cent. 0.006 M aluminum tartrate). This effect diminishes rapidly with lower lime nutrients, until at the point at which the proportions are 50 per cent. of 0.006 M lime and 50 per cent. of 0.006 M aluminum tartrate the results are almost the same with respect to relative growth as if no lime were added at all (table XVI). The same behavior applies, only to a more limited degree, to the case of relative length of tops. With respect to total dry weight the mixture finally indicates over a range a condition of apparent additive effect with respect to toxicity, the mixture promoting a smaller relative growth than the single toxic salt (fig. 4 *A, B, C*).

Mixtures of aluminum tartrate and potassium hydroxide.—When potassium hydroxide is mixed with aluminum citrate in different proportions the growth is retarded markedly except in one proportion. This proportion is approximately 30 per cent. of 0.006 M aluminum tartrate and 70

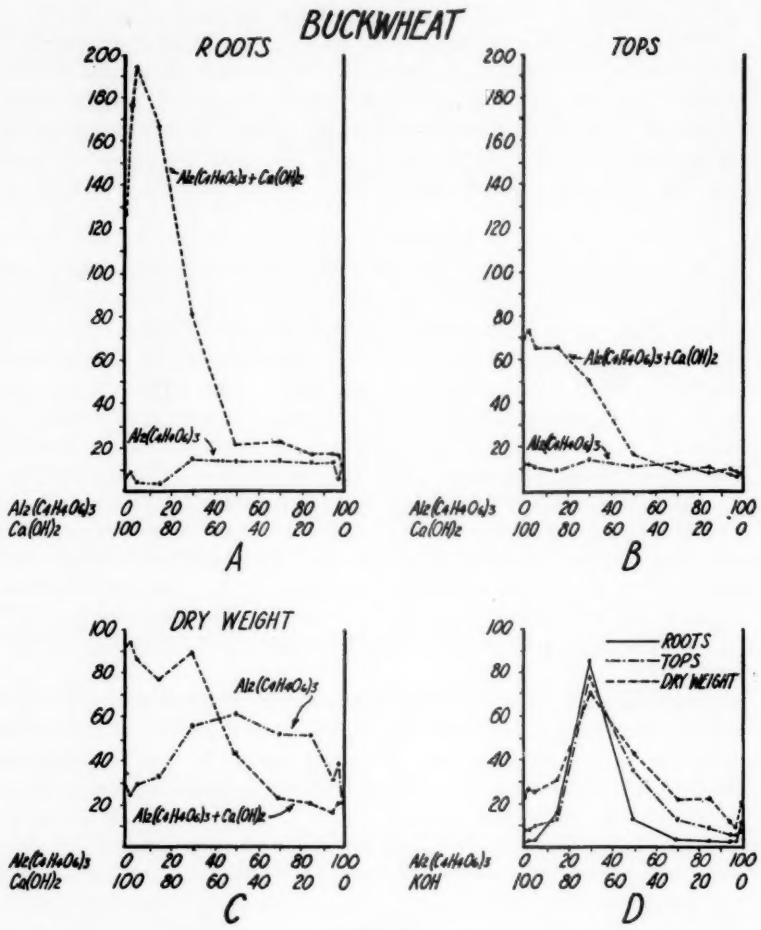

FIG. 4

A: Elongation of roots of buckwheat seedlings grown in single salt solutions of aluminum tartrate, and in mixed solution of aluminum tartrate and calcium hydroxide.

B: Elongation of tops of buckwheat seedlings grown in single salt solutions of aluminum tartrate, and in mixed solution of aluminum tartrate and calcium hydroxide.

C: Total dry weight of buckwheat seedlings grown in single salt solutions of aluminum tartrate and in mixed solutions of aluminum tartrate and calcium hydroxide.

D: Elongation of roots and tops in addition to total dry weight of buckwheat seedlings grown in mixtures of aluminum tartrate and potassium hydroxide. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

per cent. 0.006 M potassium hydroxide. In all others the growth falls off markedly. For seedlings growing in water solutions this probably indicates an optimum pH. The potassium evidently does not have much effect in alleviating the toxicity of aluminum, and at the higher pH the condition is too alkaline for the plants to thrive. The optimum pH is almost 9 (table XVII). This optimum does not nearly approach the optimum for mixtures of the aluminum salt and lime with respect to the degree of relative growth (fig. 4 *D*). Mixtures of lime and aluminum salts for all plants investigated have wide and high limits of growth, while potassium mixtures at nearly the same pH have narrower and lower limits of relative growth.

Summary

1. Organic aluminum compounds exert a distinct toxic effect on the plants of corn, soy beans, and buckwheat. The toxicity increases with increased concentration.

2. The toxicity for the three plants could be most effectively overcome by calcium hydroxide. Calcium nitrate could be used as an antagonist of aluminum, but not as effectively as the calcium base.

3. Potassium hydroxide was less effective in counteracting the toxicity of aluminum than was calcium hydroxide. It apparently served to neutralize the acidity of the aluminum salt.

4. The favorable hydrogen ion concentration is not the same for all solutions. The optimum pH varies with the components and their proportions present in the solution.

5. Comparing the hydrogen ion concentration of the organic acids with similar hydrogen ion concentrations of the corresponding aluminum salts of these acids, it is shown that at appreciable concentrations the aluminum salt suppresses growth to a markedly greater extent than does the acid.

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LINKAGE BETWEEN OUTPUT OF ELECTRIC ENERGY BY POLAR TISSUES AND CELL OXIDATION

H. F. Rosene and E. J. Lund

(WITH SIX FIGURES)

Introduction

Evidence is presented in this report which it is believed establishes the following facts: (*a*) A quantitative relation exists between cell oxidation and the continuously maintained E.M.F.'s in the root of the onion, *Allium cepa*. (*b*) Equal change in oxygen concentration around different regions of the root tip produces an unequal change in E.M.F.; in this way the characteristic distribution of E.M.F.'s per unit length of root and therefore the pattern of the correlation currents in the root are modified in a characteristic manner by change in oxygen tension. (*c*) The velocity of oxygen consumption per unit volume of tissue is greatest in the region of active cell division, and the velocity of oxidation in this region is also changed to a greater degree by the same change in oxygen tension than in other regions of the root tip. This fact, which appears to have an important bearing upon the nature of bioelectric currents, was previously discovered in the *Obelia* stem (**17**). The experiments supply the connecting link in the evidence for the validity of the theory that the continuously maintained E.M.F. in a polar cell is that of a redox system maintained in state of flux equilibrium as defined elsewhere by one of us (**17**).

There is a correspondence of morphological, functional, and electrical polarity along the root axis of *A. cepa*. In the unstimulated uninjured condition, the region of active cell division is invariably electropositive in the external circuit to more basal regions; and the distribution of potentials is in general characteristic but in detail specific for each root. For illustrations of the exact distribution of these potentials the reader is referred to the curves in the paper by Lund and Kenyon (**13**). Under constant external conditions, spontaneous fluctuations in electric polarity occur and the distribution of E.M.F. per unit length varies from time to time (**20**). The first evidence that cell respiration and electric potentials on the root are linked phenomena was furnished by Lund and Kenyon (**13**). They found that the greatest reduction of methylene blue per unit length occurs in the region of high positive potential, where active cell division takes place, and the same region also produces more CO_2 per unit length. A corresponding distribution of the concentration of sulphydryl groups per unit length has been demonstrated; the region of active cell division exhibits the most intense color when the nitroprusside test is applied (**17**).

The present investigation was limited to experiments on not more than 7 mm. of the distal end of the root and for the most part to 6 mm. or less. This region of 6 ± mm. will be designated the "root-apex" to facilitate presentation. The occurrence of progressive cellular and tissue differentiation from primordial meristematic cells to permanent tissue is a salient feature of the polar structure of the root-apex. In roots 40 to 65 mm. in length, the root cap is not over 0.35 mm.; the region of active cell division, 1 to 1.5 mm.; and the zone of cell enlargement and cell maturation, 1 to 2 mm. Permanent tissues are present in the fifth and sixth millimeters. In recent detailed experiments performed in this laboratory by A. H. HANSZEN, it has been observed that the greatest amount of elongation takes place within the third millimeter. Although the transitional stages are not definitely delimited, the cells in the distal third (2 ± mm.) of the root-apex are relatively young, those in the middle third (2 ± mm.) are approaching maturity, and those in the proximal third (2 ± mm.) are relatively old. In this paper attention will be focused upon the electrical behavior of these three regions in relation to concentration of oxygen.

Methods

Since the material used in the experiments involved only a total of 6 or 7 mm. of the root tip, and since the conditions for the experiment often demanded that oxygen or other gases be accurately applied to a root region 1 mm. in length, without mechanical stimulation and any apparent change in humidity or temperature, a new apparatus and technique was developed. This has shown itself to be an appreciable improvement over other procedures. The apparatus is susceptible of modification for particular purposes and has been found so satisfactory that a detailed description seems justified. Recent adaptations of the apparatus permit very precise procedures of manipulation and measurements on certain single cells under ideal conditions.

The electrode chamber is illustrated in figure 1. A removable glass cover (*H*) fits closely to a rubber cushion (*P*), which is cemented to a block of bakelite (*C*) that serves as the floor of the chamber. The base (*C*) has four large and four small perforations through which connections penetrate to the outside. A rod (*F*) which passes through one of the large perforations in the rear is attached to a short piece of glass tubing that insulates and serves as a support for the adjustable swivel attachment (*K*). The onion bulb is held and protected by a paraffined cork ring which fits a hole in this attachment. The other opening (*M*) in the rear of the base furnishes means by which additional apparatus (fig. 2, B) may be inserted and connected to the outside for manipulation. Capillary tubes (*T* and *T'*), attached to electrode cups (*D* and *D'*), pass through the large open-

ings in front. The four large perforations are lined by rubber finger-stalls (*N*) which keep the chamber airtight and through which the movable connections penetrate. Leads from isoelectric zinc-amalgam zinc-sulphate electrodes (*E* and *E'*) pass through insulated glass tubes in two of the small perforations in the base, and glass tubes (*I* and *O*) which line the other two provide for the inlet and outlet of gases from external sources. During each experiment the chamber is lined with wet filter paper and a film of water is maintained at its base. The atmosphere of the chamber can be brought to a necessary complete water saturation and can be completely isolated from the exterior.

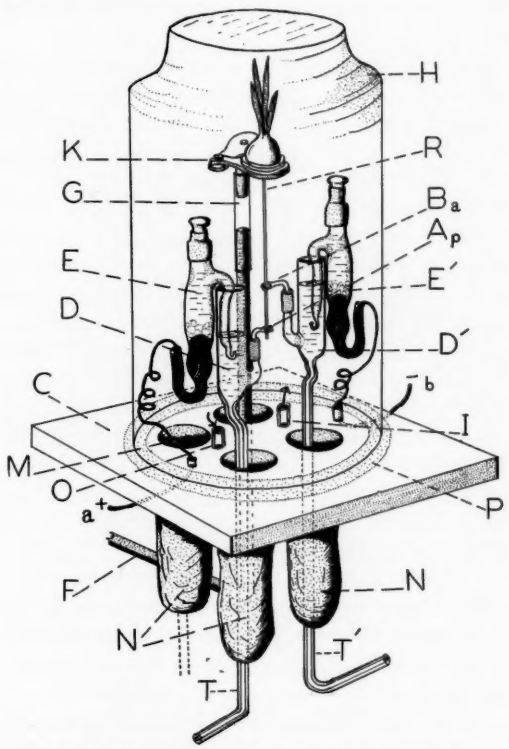

FIG. 1. Electrode chamber (description in text).

The connections outside of the electrode chamber are illustrated in figure 2, A. The bakelite base is firmly attached to a rack and pinion stand (*T*). Three micromanipulators (*A, B, C*), each of which has a three-way movement, provide adjustable support for the root holder (*K*) and the two

electrode cups (*D* and *D'*, fig. 1). These electrode cups are filled respectively from the connecting reservoirs (R_1 and R_2) through corresponding three-way stopcocks (S_1 and S_2) which are also connected to tubes for draining and washing the cups. Gases may be introduced into the electrode chamber by connecting the inlet tube (*I*) to the appropriate stopcock (S_3). They may be stored in the reservoirs (E_1 and E_2) of about 75 cc. capacity which are connected to the mercury leveling cups (G_1 and G_2) and passed into the electrode chamber by adjusting the height of the cups, each of which is supported by a rack and pinion stand not illustrated in figure 2; or by appropriate manipulation of the connecting three-way stopcocks they may be admitted directly into the electrode chamber without being stored in the reservoirs.

Gases are applied to or withheld from a specific region by means of a hollow glass jacket (fig. 2, B). The body of the jacket (*V*) consists of a short piece of glass tubing 4 mm. in length. Small glass coverslips (*O*) are cemented to the top and bottom and small holes (*I*) in each just permit passage of the root (*R*) without its touching the sides of the holes. Glass capillary tubes (*P*) sealed to two holes in the side of the jacket provide for the inlet and outlet of the gases and are attached to a glass rod (*W*) which serves as a support. The jacket is inserted into the electrode chamber through an opening (*M*, fig. 1) in the base and it is manipulated by an additional three-way micromanipulator not illustrated. By means of this micromanipulator and a horizontal microscope the jacket can be accurately adjusted to any position without stimulating or injuring the root. With the jacket in place around the root (*R*), the openings (*I*) at the top and bottom are sealed with a small drop of water or paraffin oil. In this way the segment of the root inclosed by the jacket is completely isolated from the atmosphere in the electrode chamber and gases can be introduced into and removed from the jacket under complete control by connecting the inlet tube of the jacket directly to the proper stopcock (S_3 in fig. 2, A). With the jacket in place, the root can be exposed to a gas by either of two methods: (1) introducing the gas directly into the jacket around a particular segment of the root and not into the electrode chamber; or (2) passing the gas into the electrode chamber and not into the jacket, which in that case is filled with moist air.

Electrode contacts are made by the small glass claw projections (*K* in fig. 2, B) of the electrode cups which are filled with the medium in which the roots grow. The adjustments of the positions of the contacts, which can be accurately controlled by the micromanipulators, are made under a horizontal microscope which is fitted with an ocular micrometer for measurements. From the preceding description it is obvious that the apparatus permits complete and accurate control of all manipulations and environmental conditions of the root.

All experiments were carried out at room temperature which did not vary more than 0.5° C. throughout any one experiment. The onions were grown in tap water and used when the roots were 40 to 50 mm. in length. A different root was used in each experiment. All roots but one were removed from the bulb, care being taken not to stimulate this root when it was placed in position in the electrode chamber. Potentials were measured with a Compton electrometer. In general, the same procedure was followed in each experiment. When adjustments of the positions of the jacket and the contacts on the root had been made, the preparation was left undisturbed for a short period. Observations were then made of the electric behavior of the root during an initial period of exposure to the vapor-satu-

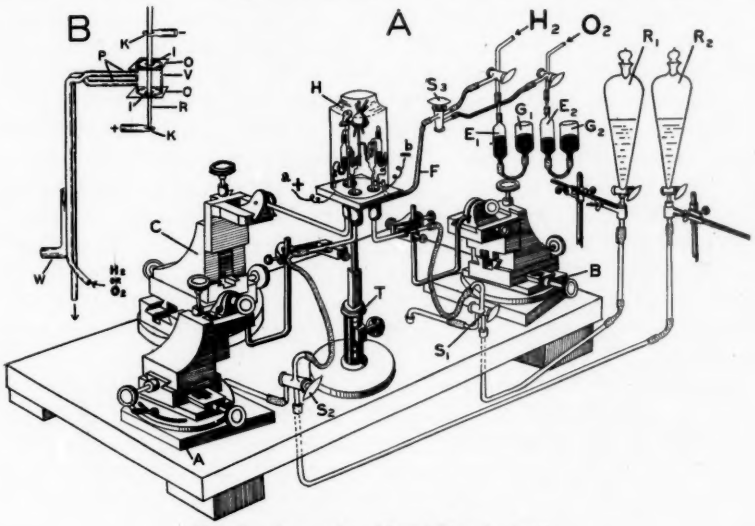

FIG. 2. Apparatus (description in text).

rated atmosphere of the electrode chamber. These readings were continued during the following two successive periods, when (*a*) hydrogen was passed through the electrode chamber or the jacket, and (*b*) oxygen was passed through in a similar manner. Before entering the chamber or the jacket, the hydrogen and oxygen were saturated by passage through distilled water. A uniform and controlled rate of flow of each gas was maintained in all the experiments by suitable pressure gauges attached to the gas tanks. The flow of gas, as such, did not produce any visible effect on the root and did not appear to affect the E.M.F. The root tissue was in no way injured by the experimental procedure and normal growth continued when the roots were replaced in tap water.

Effect on E.M.F. of the root-apex produced by change in oxygen concentration at different regions between electrode contacts

1. CHANGE IN E.M.F. WHEN THE APICAL END (1.5 MM.) IS EXPOSED TO (*A*) HYDROGEN AND (*B*) OXYGEN.—Figure 3, A shows the position of the jacket and electrode contacts in a series of three duplicate experiments on roots of three different bulbs (curves 1, 2, 3). The basal grounded electrode contact (–) was made at the upper aperture of the jacket and the jacket was filled with moist air.[1] The E.M.F. of the region between the electrodes was allowed to come to a constant value in air during a 10-minute period as shown in curves 1, 2, and 3, figure 3, A. Hydrogen gas saturated with water vapor was then applied to all of the root and bulb (except that segment inclosed by the jacket) through the connecting tubes (*I* and *O*, fig. 1). Since the results of all the experiments were remarkably similar, even in detail, only three curves (1, 2, 3, fig. 3, A) are presented. Each curve shows that the removal of oxygen by hydrogen around the apical 1.5 mm. of the root rapidly diminished the electric polarity of the root included between the electrodes. Curves 2 and 3 show a complete reversal of polarity. The initial large drop in potential was followed by a smaller rise, the magnitude and duration of which was specific for each root. At the end of the period in hydrogen the E.M.F. was lower than at any time when in air. Deprived of available oxygen by the exposure to hydrogen, the apical end of the root-apex did not maintain its characteristic high positive potential and consequently the electric polarity was considerably reduced in magnitude.

Replacement of hydrogen by oxygen saturated with water vapor produced an abrupt rapid increase of the E.M.F. of such great magnitude that the potential difference not only reached but very often exceeded its former value in air, *exhibiting the typical rebound curve observed in experiments on frog skin and Douglas fir* (**15, 18**). Curves 1 and 3, figure 3, A, illustrate the fact that the E.M.F. was often maintained at a new high level in oxygen. The effect of removal of oxygen was promptly and completely reversible.

The curves show that a definite quantitative relationship exists between cell oxidations at the apical end of the root-apex and the magnitude and orientation of electric polarity.

2. CHANGE IN E.M.F. WHEN THE BASAL END (1.5 MM.) IS EXPOSED TO (*A*) HYDROGEN AND (*B*) OXYGEN.—The experimental arrangement is illustrated in figure 3, B. Four mm. of the root-apex were inclosed by the air-

[1] When both apical and basal contacts were made at the water menisci in the apertures of the air-filled jacket under the same experimental conditions, little or no change in E.M.F. was produced when the part of the root outside of the jacket was subjected to change in oxygen concentration.

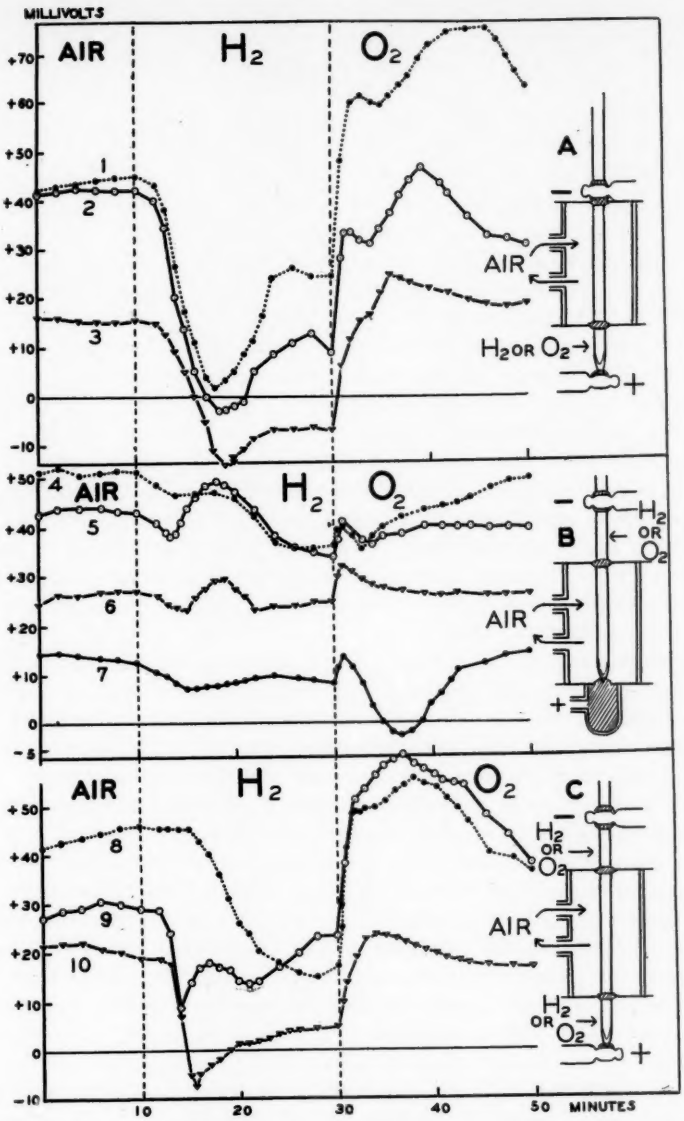

FIG. 3. Effect of change in oxygen concentration at apical and basal ends of root-apex.

A: Effect of (*a*) hydrogen, and (*b*) oxygen at apical end only, on total E.M.F. of root-apex. Curves 1, 2, and 3 obtained from three duplicate experiments on three different roots. Diagram A shows positions of electrode contacts and gas jacket on root-apex.

B: Effect of (*a*) hydrogen, and (*b*) oxygen at basal end only on total E.M.F. of root-apex. Curves 4, 5, 6, and 7 obtained from four duplicate experiments on four different roots. Diagram B shows positions of electrode contacts and gas jacket on root-apex.

C: Simultaneous effect of (*a*) hydrogen, and (*b*) oxygen at both apical and basal ends of the same root-apex on total E.M.F. Curves 8, 9, and 10 obtained from three duplicate experiments on three different roots. Diagram C shows position of electrode contacts and gas jacket on root-apex.

filled jacket and 1.5 mm. at the proximal (basal) end were exposed to change in oxygen concentration. Curves 4, 5, 6, and 7, figure 3, represent typical results and should be compared with curves 1, 2, and 3 of figure 3, A obtained under similar experimental conditions. The only essential difference is that in the two sets of experiments different ends of the root-apex were exposed to the change in oxygen concentration. A comparison of the two sets of curves reveals an unequal effect of equal change in oxygen concentration at apical and basal ends. Passing hydrogen through the electrode chamber when the basal end was exposed did not produce the marked depression of electric polarity which occurred when the apical end was similarly exposed. The electric polarity of the root-apex was never observed to become inverted by an oxygen deficit at the basal end only. Admission of oxygen to the chamber produced a relatively small rebound effect. The relatively small magnitude of this rebound effect in the basal region is comparable to the correspondingly small rebound effects observed in basal regions in the Douglas fir, when the electric polarity is changed by change in temperature (**18, 19**). It should be observed that curves 1, 2, and 3 are quite uniform in appearance. This statement does not apply in the same degree to curves 4, 5, 6, and 7.

If the apical and basal ends of the same root were simultaneously exposed to (*a*) hydrogen and then (*b*) oxygen under the same conditions which obtain in the above sets of experiments, the observed unequal effect of equal change in oxygen concentration on apical and basal ends of different roots should appear. Since it has just been shown that the effect on the apical end was noticeably uniform in character and much greater in magnitude than the effect on the basal end, it would be logical to anticipate results which would give curves similar to curves 1, 2, and 3, figure 3. A small increase in potential difference in hydrogen as shown in curves 5 and 6 and a small decrease in potential in oxygen as illustrated by curve 7 would definitely modify the characteristic form of such curves as 1, 2, and 3 because the principle of summation of cell E.M.F.'s applies in each measurement. When this procedure was followed the expected results were fully realized, as shown in curves 8, 9, and 10 of figure 3, C and what follows.

3. CHANGE IN E.M.F. WHEN THE APICAL AND BASAL ENDS OF SAME ROOT-APEX ARE SIMULTANEOUSLY EXPOSED TO (*A*) HYDROGEN AND (*B*) OXYGEN.—Four mm. of the root tip were inclosed by the moist air jacket and 1.5 mm. on either side exposed as shown in the diagram (fig. 3, C). Similar curves were obtained in all the experiments, each of which was obtained from a different root. Only three curves are given, curves 8, 9, and 10, figure 3. They convincingly show the unequal effect on the E.M.F. by equal change in oxygen concentration at apical and at basal ends of the

same root-apex. There is a pronounced similarity between curves 8, 9, and 10, and curves 1, 2, and 3 (fig. 3). Evidently in both sets of experiments the general character of the curves can be attributed to the greater change which took place in the highly electropositive apical end. A temporary complete reversal of polarity is exhibited by curve 10. The conspicuous rebound phenomena observed in oxygen in the first set of experiments reappeared. Its modification in curve 10 may be due to a flattening effect produced by a change in an oppositely oriented E.M.F. in the basal end as exhibited by the dip in curve 7, figure 3. In this connection it is of special theoretical interest to note the uniform appearance of (*a*) the relatively large depression of E.M.F. in hydrogen and (*b*) the conspicuous rebound phenomena in oxygen, whenever the *apical end* was exposed.

4. CHANGE IN E.M.F. WHEN AN INTERMEDIATE REGION BETWEEN APICAL AND BASAL ENDS IS EXPOSED TO CHANGE IN CONCENTRATION OF OXYGEN.—The position of the jacket as shown in figure 4, D is similar to that illustrated in figure 3, C, except that connections were made from the hydrogen and oxygen reservoirs (E_1 and E_2, fig. 2) to the glass jacket around the root. By careful vertical movement of the rack and pinion stand which supported the mercury in the leveling cups (G_1 and G_2, fig. 2), a constant flow of gas through the jacket was maintained at such a rate that the mechanical conditions of gas flow as such produced no effect on the E.M.F. The lengths of the apical and basal segments, which were outside of the jacket, were not equal in all the experiments; they varied from 0.8 to 1.8 mm.

Since a quantitative relationship between changes in oxygen concentration at the apical and basal ends and the E.M.F. of the root-apex was demonstrated above, and since it has been shown by MARSH (**20**) that the principle of summation of cell E.M.F.'s applies to the first 30 mm. of the root tip, it is reasonable to expect a change in E.M.F. with change in oxygen concentration around the intervening zone (fig. 4, D) under conditions corresponding to those which prevailed in the preceding sets of experiments. Furthermore, it is to be expected that the magnitude and direction of effect will depend not only upon the length of the intermediate exposed zone, but also upon its distance from the distal and proximal limits of the root-apex. For example, if the segments X and Y outside the jacket in figure 4, D are relatively short (*i.e.*, 0.5–1 mm.), the magnitude of the effect would be relatively greater than if a longer region at each end were isolated, because some of the apical tissue would be in the intervening zone inclosed by the jacket, and it has already been found that greater effects are produced in the apical cells.

Curves 1, 2, and 3, figure 4, are representative of the results from eighteen experiments on different roots. The corresponding lengths of X

and Y, which were equal for each root, were respectively 1.8, 0.8, and 1.3 mm. Curve 1 shows that the potential difference was steadily drifting to an increase in E.M.F. during the period in air, but in hydrogen this drift was

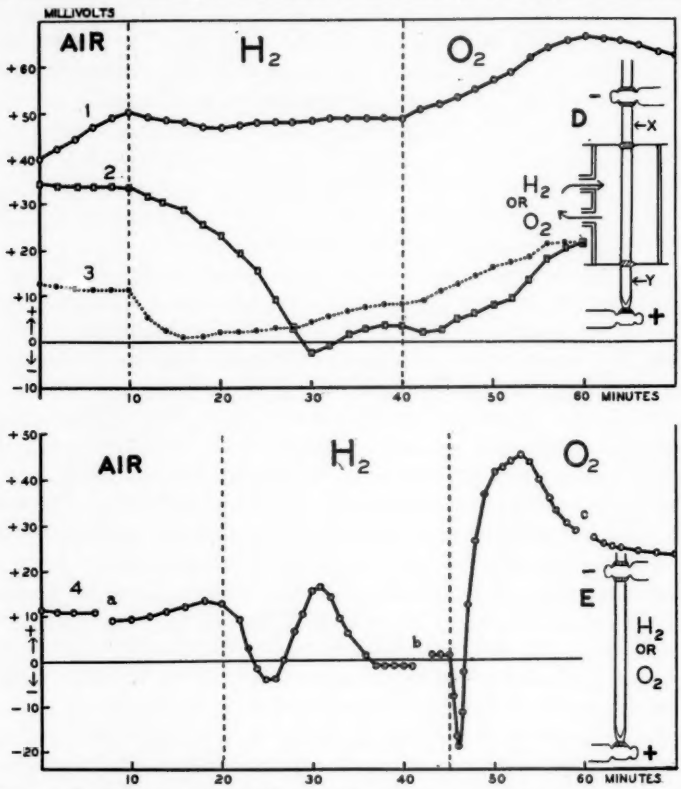

FIG. 4. D: Effect of application of (*a*) hydrogen, and (*b*) oxygen at an intermediate region of root-apex on total E.M.F. Curves 1, 2, and 3 obtained from three similar experiments on three different roots. Positions of electrode contacts and gas jacket shown by diagram D. X and Y are segments of the root exposed to air.

E: Effect of (*a*) hydrogen, and (*b*) oxygen applied simultaneously at all regions of root-apex on total E.M.F. Positions of electrode contacts shown by diagram E. Results shown by curve 4. The breaks (*a*, *b*, and *c*) in curve 4 are the intervals during which the curve of distribution of E.M.F. per unit length of root was determined in (*a*) air, (*b*) hydrogen, and (*c*) oxygen respectively.

opposed in some way and the curve flattened. The drift again manifested itself in oxygen. Curve 2 shows the greatest change. An inverted polarity appeared temporarily in hydrogen and a steady recovery in oxygen. The

relatively greater effects shown in curve 2 are undoubtedly associated with the exposure of a greater portion of the sensitive apical end to change in oxygen concentration. To some extent there is a general similarity of curves 1, 2, and 3, figure 4, to curves 4 and 7, figure 3; but the uniformity of effect on total E.M.F., which is expressed by the relatively large decrease in potential difference in hydrogen and the relatively large increase in oxygen (with its accompanying rebound phenomenon as represented by curves 1, 2, and 3, and curves 8, 9, and 10, in figure 3), is absent. The explanation of the apparently small effect of change in oxygen tension in this set of experiments will be evident from what follows.

Unequal effect on E.M.F. of root-apex produced by simultaneous equal change in oxygen concentration at all regions of root-apex

An inspection of the curves in figures 2 and 3 in LUND and KENYON'S paper (**13**) and in figures 3, 4, and 7 of MARSH'S paper (**20**), which represent the distribution of E.M.F. over longer regions than the root-apex itself, shows that the occurrence of a *single unidirectional* gradient of E.M.F. is the rule in the length of 6 ± mm. designated in this paper as the root-apex. Many similar determinations of the distribution of potential over the root made by the present writers confirm this conclusion.

The magnitude and form of the curve representing the single gradient in the root-apex are specific for each root. Six such gradients of potential differences in six different roots are illustrated by curves 1a, 2a, 3a, 4a, 5a, and 6a in figure 5. The gradients were determined in the usual manner by moving the electrode contact at the apex by increments of 1 mm. toward the basal contact. In the roots corresponding to curves 4a, 5a, 6a, the basal electrode contact was fixed at a position 6 mm. from the tip; in the roots corresponding to 2a and 3a it was fixed at 5 mm.; and to 1a, at 4 mm. from the tip. Individual variations in the single gradient characteristic of the root-apex are apparent. Curve 5a shows that a point 2 mm. from the tip is electronegative to relatively more basal points, thus producing a small dip in the gradient which indicates the presence of inverted component electric polarities. Similar inverted regions appear in some of the other curves. The form of the gradient would probably appear in more detail if readings were made at intervals of 0.2 mm. instead of 1 mm. The observed gradient of potential of the root-apex is a resultant E.M.F. which expresses the algebraic sum of the E.M.F.'s of individual cells. This does not exclude the possibility of a complex pattern of cellular E.M.F.'s within the root-apex which probably involves series and parallel arrangements of the cells.

The uniformity of results observed whenever the apical end was exposed to changes in oxygen concentration, and the variability of the curves obtained when either the basal end or the intervening region alone was simi-

larly exposed, are related to detailed variations of the distribution of E.M.F. per unit length of the root. A sufficiently large diminution of an inverted component E.M.F. would appear as an increase in the resultant E.M.F. of the gradient. The facts established by the preceding sets of experiments lead to the conclusion that if all the regions of the root-apex were simultaneously exposed to equal change in oxygen concentration, unequal change in regional E.M.F.'s and in the resultant E.M.F. would appear; and furthermore, the generalized nature of the composite curve which would result under these conditions would be determined by the relatively greater changes in apical component E.M.F.'s. Figure 4, E shows the arrangement in a series of experiments of this nature.

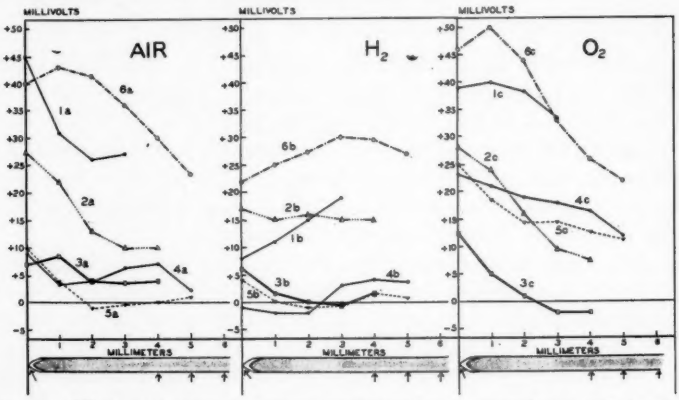

FIG. 5. Effect of change in oxygen concentration on magnitude and form of the curve of distribution of E.M.F. over the length of root-apex in (*a*) air, (*b*) hydrogen, and (*c*) oxygen. The curves were obtained from six experiments on six different roots. Curves 1a, 2a, 3a, 4a, 5a, and 6a, when the electrode chamber (cf. fig. 1) was filled with moist air; curves 1b, 2b, 3b, 4b, 5b, and 6b, when hydrogen was passed continuously through the electrode chamber; and curves 1c, 2c, 3c, 4c, 5c, and 6c, when oxygen was passed continuously through the electrode chamber. The diagrams of the roots below the curves are drawn to scale on the abscissa. Arrows at the apex indicate position of positive electrode. The three arrows at the base indicate the exact positions of the negative electrode which was stationary at different distances from the tip in the different experiments. Corresponding to curves 1a, 2a, and 3a; and 4a, 5a, and 6a, the basal electrode was fixed as shown at 4, 5, and 6 mm. respectively from the tip.

The distance of the basal electrode contact from the contact at the tip was not the same in all the experiments. Hydrogen and oxygen were passed through the electrode chamber. The curves for different roots were essentially alike. Curve 4, figure 4, is typical. In this experiment the electrode contacts were 6 mm. apart. In hydrogen an initial relatively large drop of total E.M.F. was followed by a rise and finally a decrease,

with the potential difference maintained at a low level. Recovery occurred when pure oxygen was substituted for hydrogen and the conspicuous rebound effect appeared (*cf.* with curves 1, 2, 3, and 8, 9, 10 of figure 3). It is evident that equal and simultaneous change in oxygen concentration changes the localized E.M.F.'s unequally. This would necessarily modify the distribution of E.M.F.'s per unit length which constitute the resultant gradient. The gradient of electric polarity would be different in an atmosphere of air, an atmosphere devoid of oxygen, and an atmosphere of pure oxygen. This conclusion is tantamount to the statement that the oxygen concentration around the root determines to a large degree the characteristic magnitudes of E.M.F.'s per unit length.

In each experiment, when the root-apex exhibited a relatively stable potential difference in (*a*) air, (*b*) hydrogen, and (*c*) oxygen, the distribution of potential difference was determined by the customary method of moving the apical electrode. The position of the breaks (*a, b,* and *c*) in curve 4, figure 4, indicates the intervals during which the gradients shown by curves 4a, 4b, and 4c in figure 5 were determined. Curves of the gradients determined in five similar experiments on five different roots are also given in figure 5. Curves 4a, 4b, and 4c show that the gradient was inverted in hydrogen with the apical end electronegative to more basal regions, and that it was displaced to a lower level of potential difference; in oxygen the gradient not only righted itself but also manifested a greater total E.M.F. The dip in each of the curves 4a and 4b, which indicates the existence of components with inverted polarity, disappeared in curve 4c. Gradients corresponding to curves 1a, 1b, 1c, and 6a, 6b, 6c displayed changes practically identical with those exhibited by curves 4a, 4b, and 4c. In hydrogen each gradient was inverted, the apical end became electronegative to relatively more basal regions, and the whole gradient shifted to a lower value; in oxygen each gradient righted itself and curve 6c shows that the corresponding gradient manifested a higher E.M.F. Gradients corresponding to curves 2a and 3a were flattened in hydrogen and became steeper in oxygen with the apical end more highly positive than at any previous period. All the curves in figure 5 obtained by this procedure show that the component parts of each gradient were affected unequally by equal change in oxygen concentration, and that the component E.M.F.'s associated with the tissues in the apical end were displaced to a relatively greater degree than were component E.M.F.'s in the other tissues of the root-apex. An atmosphere of hydrogen tends to make the ends of the gradient more alike; oxygen more unlike. Hydrogen tilts the gradient in a direction opposite to that characteristic of the root-apex in air; oxygen tilts it back to a steeper form. The form of the gradient at any one instant is determined by the concentration of oxygen at that instant.

The curves in figure 5 also throw light on the nature of the phenomena which determine the character of curve 4, figure 4. The initial drop in curve 4 (in hydrogen) is explained by the fact that introduction of hydrogen into the electrode chamber depressed the local polarity potentials in the apical region more than the local polarity of other regions. The high positive potential of the apical end disappeared and the apical end became temporarily electronegative to relatively more basal regions. The rise in the curve in hydrogen is due to the depression of inverted constituent E.M.F.'s and the relatively slow depression of basal components. Finally depression of all the component E.M.F.'s and the relatively greater depression of apical components produced a flattened or inverted gradient and hence a second drop in the curve in hydrogen as shown. In the absence of such definite information as in the foregoing, the initial inverted bayonet-like part of the curve which occurs when oxygen is admitted to the chamber might be explained by either (*a*) a sudden increase in positivity at the basal end, or (*b*) a sudden decrease in the apical end. The results indicate that (*a*) is probably the correct interpretation. Curve 4 shows that the duration of this abrupt change was short. It was followed by a greater increase in positivity in the apical regions than in the basal regions, replacing the gradient to its previous slope in air and manifesting the characteristic rebound. During the rebound the slope assumed its greatest value. At the end of the period in oxygen it was less steep but as a rule the slope was greater than that exhibited by the gradient of the root in air, as the curves in figure 5 show.

The results from this set of experiments, exposing the whole root-apex to change in oxygen concentration, corroborate the conclusions based upon the preceding sets of experiments, in which local regions only were exposed to change in oxygen tension. The effects on the young tissue at the apical end are relatively and absolutely so large in magnitude that they determine the character of the resultant polarity potential of the whole. This illustrates what may properly be called an electrical dominance of the apex.

Absence of significant change in total E.M.F. when oxygen concentration is changed in region outside of electrode contacts

In several of the preceding sets of experiments the hydrogen and oxygen were passed directly into the electrode chamber exposing the whole root and bulb. A sixth set of experiments was therefore carried out in order to determine whether the observed modifications of E.M.F. in hydrogen and oxygen were linked with changes in parts of the root not included in the region between the electrode contacts.

The position of the contacts and jacket is shown in figure 6 (diagrams F and G). The gases were passed through the jacket. Curves 1 and 2 in figure 6

show the results when the chamber was placed as illustrated in the diagrams (F and G). The curves show no significant changes in E.M.F. in hydrogen and oxygen; neither did curves obtained when the periods of exposure were increased to 1-hour intervals. It is evident that the observed changes in E.M.F. concurrent with changes in oxygen concentration were due to changes in E.M.F. in the cells at and between the electrode contacts, and not to changes in neighboring cells. The experiments therefore indicate that the local changes in E.M.F.'s produced by change in oxygen tension are not transmitted in the ordinary sense of conduction of excitation, within the period of the experiments.

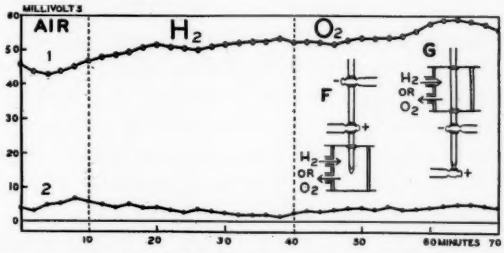

FIG. 6. Absence of effect of change in oxygen concentration when gas jacket is placed outside of electrode circuit. Curve 1 was obtained when gas jacket and electrode contacts were in the position on the root shown in diagram F; curve 2, when gas jacket and electrode contacts were in the positions shown in diagram G.

Discussion

The preceding evidence, together with previously published facts on the respiratory exchange, conclusively establishes the fact of a quantitative linkage between electric polarity and respiration in the root-tip. That region which manifests the greatest magnitude of change in its regional polarity potential is the same region which exhibits the highest positive potential, the largest output of carbon dioxide production and oxygen consumption, the greatest capacity for methylene blue reduction, the highest concentration of sulphydryl groups, and the region in which visible structural differentiation is at a minimum. These facts furnish a complete chain of evidence that morphological, functional, and electrical polarities are interrelated phenomena associated with the oxidative metabolism and with specific differences in the oxidative mechanisms of young and old tissues.

The reversible inhibition of polarity potentials in the root-apex produced by the absence of oxygen is similar to the reversible inhibition of electric polarity in the stem of *Obelia* and in frog skin produced by cyanide, ether, and chloroform (**12, 16**). In the root-apex and in *Obelia* the percentage depression of E.M.F. was greater in the apical region than in the basal region. LUND (**15**) found that the electric polarity of frog skin was

changed by equal changes in oxygen tension on the two sides of the skin. As in the root apex, an oxygen deficit decreased the potential difference and exposure to high oxygen concentration increased the difference. Reversible effects were observed in frog skin but the electric polarity was never completely inverted as in the root-apex.

The characteristic rebound in E.M.F. which occurs when (*a*) the whole "root-apex" or (*b*) the apical end only is exposed to oxygen after a period in its absence, is similar to the rebound effect which has been observed in the E.M.F. of frog skin when the oxygen concentration was changed from a low to a high value (**15**), and the E.M.F. of Douglas fir when the temperature was restored to its original value after a period of exposure to low temperature (**18, 19**). Furthermore, the rebound effect is also similar to the increase in velocity of oxidation, observed by a number of investigators, when certain tissues and organisms were exposed to oxygen after a period in its absence; nerve (**6, 5**), muscle (**5**), tomato fruits (**7**), *Planaria* (**11**), and luminous bacteria (**25, 8**). GUSTAFSON (**7**) also found that, in a given tomato fruit, the magnitude and continuance of increased velocity of oxidation when air was reintroduced into the chamber were dependent upon the length of the period of exposure to pure hydrogen or pure nitrogen. Although he makes no mention of it, his data show that the greatest increase in velocity of oxidation is associated with the younger green fruits as compared with the increase in respiratory activity of older pink or red-ripe fruits. *These rebound phenomena in the velocity of oxidation are obviously to be explained by an accumulation of oxidizable substances during the periods in absence of oxygen or in low oxygen tension, and their increased rate of removal by oxidation upon readmission of oxygen.* In such systems the $\frac{\text{Red}}{\text{Ox}}$ ratios are clearly subject to change by change in the concentration of oxygen. The general occurrence of a rebound effect in the velocity of cell oxidations and the rebound effect in the E.M.F.'s under corresponding conditions of change in oxygen tension leads directly to the conclusion that the mechanism which controls the velocity of cell oxidation also controls the magnitude of the cellular E.M.F.'s.

The gradient of the distribution of cellular E.M.F.'s per unit length, which is an expression of gradation in the rate of output of electric energy quantitatively associated with corresponding differences in velocity of oxidation and structural differences, is distinctly modified by change in oxygen tension. Under the experimental conditions which obtained in this investigation its slope was greatest in oxygen, less in air, and almost obliterated or inverted in hydrogen.

Up to the present the only complete and satisfactory explanation of the experimental results is furnished by LUND's flux equilibrium theory of bioelec-

tric currents and cell oxidation which has been fully developed in a recent paper (**17**). The continuously maintained potentials are the expression of redox states of electromotively active substances in flux equilibrium in polar cells. The observed change in ΣE_p[2] of the root-apex, with equal change in [O] at the apical end only, the basal end only, or the intervening region, is due to unequal changes in $\frac{\text{Red}}{\text{Ox}}$ ratios in the cells of these regions; and accordingly the change in Σ Ep with equal and simultaneous change in [O] at all the specific regions of the root-apex is also due to unequal changes in the $\frac{\text{Red}}{\text{Ox}}$ ratios of the local regions. The experiments show that the greatest change in flux concentrations of electromotively active substances in the cells of a specific region takes place in the young, relatively undifferentiated tissue of the apical end. The change in ΣE_p with equal change in oxygen concentration constitutes the first critical evidence that the velocity of oxidation in young tissue is actually greater than that in old tissue, and that the active mass of oxidizable substance (or system) AH_2 is greater in young tissue. This conclusion is in accordance with recent results (unpublished), obtained in this laboratory by L. M. HENDERSON, that the percentage increase of oxygen consumption in apical pieces of the root tip (*A. cepa*) was greater than the percentage increase of basal pieces in the same increase in oxygen. It is in agreement with the observations on *Obelia* by LUND (**17**) which showed that the increase in velocity of oxidation in apical halves of the stem was always proportionately greater than the increase in oxidation in basal halves caused by the same increase in oxygen concentration, and that there was a higher concentration of sulphydryl groups in the apical end. Finally, the conclusion that the effective concentration of oxidizable substance (or system) AH_2 is proportionately

[2] ΣE_p expresses the polarity potential of a polar tissue or organ. It is the algebraic sum of the polarity potentials (E_p) of the individual cells.

A simplified form of the electrochemical equation which expresses the polarity potential E_p of a single cell as given by LUND is:

$$E_p = \frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}$$

where R is the gas constant; T the absolute temperature; ln the natural logarithm; F, the Faraday equivalent; and $[A]_a$, $[A]_b$, $[AH_2]_a$, $[AH_2]_b$, $[O]_a$, $[O]_b$ the concentrations of electromotively active substances. For the polarity potential ΣE_p of a polar tissue the equation may be expanded to

$$\Sigma E_p = \left[\frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}\right]_1 \pm \left[\frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}\right]_2 \pm \left[\quad\cdots\cdots\quad\right]_3 \pm \left[\quad\cdots\cdots\quad\right]_n$$

where the subscripts 1, 2, 3, etc., denote different cells.

much greater in the apical end is supported by the recent observations mentioned above that the concentration of sulphydryl substances is distinctly more concentrated in the cells just behind the root cap. The fact that, as a rule, after an interval of 20 or 30 minutes in oxygen, the slope of the gradient which expresses the ΣE_p per unit length is greater than its slope in the initial period in air, shows that part of the surplus of oxidizable substances accumulated during the period in hydrogen is still available or that an increase in the velocity of the reaction $X \rightarrow AH_2$ occurred and more AH_2 is continuously available. Concomitant with the difference in magnitude of rebound in E.M.F. in apical and basal ends of the root-apex is the apparent unequal acceleration of the reaction $AH_2 + O \rightarrow A + H_2O$ in apical and basal ends. Both the concentration of oxygen and the concentration of AH_2 are limiting factors which determine the magnitude and orientation of electric polarity in the root-apex. The conspicuously greater accumulation of electromotively active material in the apical end indicates that the mechanism of accumulation of such a large surplus of oxidizable substances is a definite characteristic of young tissue.

Oxidation-reduction potentials are not, of course, the only means by which differences of potential are, or may be, established in living cells, and the flux equilibrium is not the only type of electrochemical equilibrium which may exist in the cell; but it is the only type thus far considered in the literature of electrophysiology which can maintain in a direct manner a continuous output of electric energy. The theoretical requirements of the electrochemical equations which express the relation of the observed maintained E.M.F.'s to the velocity of cell oxidation are given by LUND (**17**). The facts derived from the present investigation on the root tip, which is a polar tissue, specifically satisfy these requirements and therefore furnish direct evidence of the validity of the flux equilibrium concept.

The experimental results appear to be of vital significance for cell dynamics and structural differentiation. Very direct evidence for this has been found in experiments on the effect of applied electric currents, now in progress. The polar tissues of the root are constantly generating electric energy, and the flux equilibrium is a state maintained, at least in part, by the relations between the intake of oxygen and the concentration of available substances for oxidation in the individual cells. The structural differentiation of the cells and tissues has associated with it an electrical differentiation. The facts show that this is primarily a difference which depends for its significance upon the *intensity* factor of cell oxidation as expressed by oxidation-reduction potentials at oriented loci. There is an orientation of energy output in cells of polar tissues, and the observed E.M.F. expresses the algebraic summation of local forces. In low oxygen concentration the observed output of electric energy is diminished, in high

oxygen concentration it is increased. Continuously maintained oxidation-reduction potentials are an expression of metabolic activities associated with output of electric energy, and from this it may be concluded that oriented *electrometabolism* is characteristic of polar tissues.

It is well known that oxygen is necessary for the proper functioning of roots. Its absence inhibits growth, modifies cell division, and affects geotropic curvature.[3] It has been shown that the amount of available oxygen is also related to the rate of absorption of water and solutes by roots (**10, 23, 24, 9**). It is highly probable that the energy required for one or more of these oriented processes is derived to greater or lesser extent from the oriented continuous bioelectric currents in the root.

Summary

1. Evidence is presented which shows that the continuously maintained E.M.F.'s in the onion root (*Allium cepa*) are quantitatively linked with oxidative metabolism. By means of new apparatus and technique, described in the text, it was possible to measure the effect on the E.M.F. of the uninjured intact root when oxygen or other gases were applied to a root region of 1 mm. or more in length without mechanical stimulation and change in humidity or temperature. The investigation involved experiments on the distal 6 ± mm. of the root, a region designated as the "root-apex" for convenience.

2. Change in oxygen concentration at (*a*) the apical region (1.5 mm.), (*b*) the basal region (1.5 mm.), and (*c*) an intermediate region of the root-apex, respectively, changes the regional polarity potential of each region and correspondingly modifies the total E.M.F. of the root-apex in each case.

3. Simultaneous equal change in oxygen concentration at all the regions of the root-apex has an unequal effect on E.M.F. of the different regions of the root-apex. This is due to the fact that the effect in the young, relatively undifferentiated tissue of the apical end is relatively and absolutely so large in magnitude that it determines the character of the resultant polarity potential of the whole.

4. The effect of change in E.M.F. produced by change in oxygen concentration is reversible. In hydrogen the E.M.F. of a given region is diminished; in oxygen the E.M.F. is increased.

5. The slope of the gradient which represents the output of electric energy per unit length of the root-apex is different in (*a*) air, (*b*) hydrogen, and (*c*) oxygen. The gradient is flattened or may be inverted in hydrogen. Its slope is steepest in oxygen.

[3] A review of the literature previous to 1921 is given by CLEMENTS (3). Recent contributors are BOUYGUES (1), NAVEZ (21), NAVEZ and CROZIER (22), and ZIMMERMAN (26).

6. The observed changes in E.M.F. concurrent with changes in oxygen concentration were produced by changes in E.M.F. between and at the electrode contacts and not by changes outside of the electrode circuit. These facts constitute additional confirmation of the validity of the principle of algebraic summation of individual cell E.M.F.'s.

7. The results show that the velocity of oxidation is greatest in the cells actively engaged in cell multiplication.

8. The typical rebound phenomenon which is apparently produced by the accumulation of oxidizable substances in the absence of oxygen is greatest in the young (apical end) tissue.

9. The results furnish additional direct evidence for the validity of the theory that continuously maintained E.M.F.'s are generated by the redox system of the cell. The magnitude of the E.M.F. at any instant depends upon the conditions of a flux equilibrium in the process of cell oxidation.

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PHYSIOLOGY OF APPLE VARIETIES[1]

ALTON H. FINCH

(WITH SIX FIGURES)

Introduction

A better understanding of physiological factors determining the growth and fruiting habits of different apple varieties (*Malus malus*) should suggest answers to many common orchard problems. Why, for example, do trees of some varieties habitually bear biennially while trees of others bear regularly, although given the same cultural treatment? Why do trees of some varieties come into profitable fruiting five to six years after planting while others do not fruit commercially until ten to twelve years of age? Why does one variety bear regularly for one grower and biennially for his neighbor? Other orchard conditions present similar problems for practical solution.

The work described in this paper is the result of studies conducted at the University of Wisconsin during 1927, 1928, and 1929 in an effort to find physiological causes for the differences in growth and fruiting of some varieties of apples.

PREVIOUS DATA SUGGESTING AN APPROACH TO THE PROBLEM

A direct relation between growth and fruiting character has been suggested for trees of the Wealthy variety (5). Differences of growth character and fruitfulness were produced by varying the environmental conditions under which the trees were grown. Strongly vegetative trees making a long, slender growth with a high nitrogen nutrient and also weakly vegetative trees making a short, slender growth with a low nitrogen nutrient were non-fruitful. Between these two extremes of vegetative condition were trees having a relatively thick and moderately long growth which was fruitful.

Chemical analyses of the different types of growth revealed that the unfruitful strongly vegetative trees were high in nitrogen and low in carbohydrates, especially starch; that the weakly vegetative unfruitful trees were low in nitrogen and high in carbohydrates; and that the fruitful trees were of intermediate composition. Axillary and terminal buds were common on trees that were fruitful but which approached the strongly vegetative condition, while fruit bud formation was largely limited to spurs on trees that

[1] Publication authorized by the Director of the Wisconsin Agricultural Experiment Station.

approached the weakly vegetative condition. Between these two conditions fruit buds were produced in abundance laterally, terminally, and on spurs.

The cause of the variation in growth and fruiting within a variety has been thought (**2, 5, 6**) to lie in a difference in chemical composition, particularly as regards the relation of carbohydrates and nitrogen compounds. If this hypothesis is correct, it was reasoned that a difference in the formation and utilization of carbohydrates and compounds of nitrogen might account to a degree for the difference in growth and fruiting habits of apple varieties.

Materials

For convenience in studying the factors underlying their variable fruiting behavior, apple varieties were classified according to their usual bearing habits, as follows:

1. Biennially bearing varieties, in which a heavy crop tends to alternate each year with little or no crop under a rather wide range of orchard conditions.
2. Regularly bearing varieties, which tend to bear a satisfactory commercial crop each year when given relatively good cultural conditions.
3. Shyly bearing varieties, which tend to come into bearing late and which, for several years after beginning to bear, usually produce a light crop either biennially or regularly.

It is not presumed that such an arbitrary classification holds for all varieties under all conditions. Trees of any variety may be found in any one of the classes, provided the proper environmental conditions obtain. Trees of all varieties tend to come into the first class as they grow older. Cultural and climatic conditions influence markedly the class into which a variety falls.

Certain varieties were studied as representative of each class. Duchess and Wealthy were taken as typical of the biennially bearing varieties; Fameuse, Northwestern, and McIntosh of regularly bearing varieties; Spy and in some cases Liveland or Newell of shyly bearing varieties.

Rate and period of terminal growth.—The amount and period of terminal growth were obtained from trees growing in commercial orchards at Sturgeon Bay, Wisconsin. The growth curves for the biennially bearing Duchess and Wealthy, the regularly bearing McIntosh and Northwestern, and the shyly bearing Spy and Liveland were obtained from trees growing in the same orchard under similar soil and cultural conditions. Data upon the regularly bearing Wealthy and Duchess were obtained from rather heavily pruned and nitrated trees in a second orchard. Data for biennially bearing McIntosh, Fameuse, and Northwestern were obtained from trees in a third well cultivated but poorly fertilized orchard on light soil. The

biennially bearing Spy and Newell were old, lightly pruned trees growing in a fourth orchard receiving some cultivation but no fertilizer.

XYLEM FORMATION AND STARCH DISAPPEARANCE AND ACCUMULATION.—Xylem formation and starch disappearance or accumulation were observed in the current season's growth or in one- and two-year old wood of some of the trees just described.

MACROCHEMICAL ANALYSES.—Samples for macrochemical analyses consisted of the entire current season's terminal shoots from trees growing in commercial orchards at Sturgeon Bay, as previously described. Such material does not represent the highest or lowest extreme of carbohydrate or nitrogen content, but approximates that accompanying extremes of fruiting condition.

ANATOMICAL STRUCTURE.—This was studied in terminal shoots from trees of commercial orchards at Sturgeon Bay, in nursery trees the first season after being grafted and growing under uniform soil conditions in a nursery at Madison, and in yearling trees propagated on dwarf stocks growing in pot culture with controlled nutrients.

Methods

SAMPLING.—The method of sampling employed was carefully to select typical samples of the class or group being observed, rather than to sample at random or in large numbers and draw conclusions after mathematical treatment of the data. In all cases where terminal shoots were used, they were selected for a particular character or quality of growth (fig. 1), as these have been observed to be associated with fruiting performance of the variety being sampled. The character of the terminal growth differs with variety as regards length, diameter, tapering, bark color, willowyness, brashness, length of internodes, and size of terminal leaves. In sampling the nursery trees and those growing in pots, care was taken to obtain samples typical of the variety or plot, selections being made on the basis of growth character.

MEASUREMENT OF TERMINAL GROWTH.—Records of the rate of growth of the terminal shoots were obtained by measuring the length of the terminal shoot about every five days during the growing season. Data were taken in 1927 and 1928 but only the 1928 results are presented, as they are typical of the two seasons.

XYLEM FORMATION AND STARCH DISAPPEARANCE AND ACCUMULATION.—Samples from terminal shoots and one- and two-year old wood were collected at about five-day intervals during the spring of 1927 and 1928 at Madison and during the spring and summer of the same years at Sturgeon Bay. These were stored in formalin-alcohol-acetic acid solution (100 cc. of 50 per cent. alcohol, 2–3 cc. of acetic acid, and 7 cc. of 40 per cent. forma-

FIG. 1. Typical shoots of various fruiting classes.

A. Fruitful terminal shoots of biennially bearing trees. Left, Duchess; right, Wealthy. These shoots tend to have large terminal leaves arising at wide angles, long terminal internodes, and a uniformly thick diameter.

B. Terminal shoots of regularly bearing trees. Left, Fameuse; right, McIntosh. These shoots tend to have medium sized terminal leaves, moderately long terminal internodes, and a medium diameter which tapers somewhat.

C. Terminal shoots of shyly bearing trees. Left, Spy; right, Newell. These shoots tend to have rather short terminal internodes, small terminal leaves arising at a sharp angle, and a small tapering diameter.

D. Unfruitful terminal shoots of biennially bearing trees. Left, Duchess; right, Wealthy. These shoots tend to have short terminal internodes, small terminal leaves arising at sharp angles, and a slender diameter.

lin). Freehand transverse sections were subsequently made: (1) at a point usually in the third internode from the tip; (2) at the middle of the shoot; and (3) at the base of the shoot. For the studies involving two-year old wood similar sections were made at corresponding points. The sections

were stained with a solution of iodine in potassium iodide and mounted in a glycerin solution of the same to render the reaction somewhat permanent. The width of secondary xylem was measured in units of an eyepiece micrometer and recorded together with the estimated amount of starch. Starch storage may occur throughout the pith parenchyma but is usually more pronounced in the outer areas. It was observed in xylem and ray parenchyma and in phloem and cortical parenchyma. The amount of starch present was estimated from a consideration of the relative proportion of parenchyma cells of each tissue containing it, and also from the relative abundance with which it occurred within such cells.

MACROCHEMICAL ANALYSES.—Material for chemical analyses was collected on August 12, 1927, and July 27, 1928, at Sturgeon Bay, Wisconsin. On these dates, fruitfulness of the shoots might be expected to be related to their composition, if such relation exists, for blossom primordia were first observed during the early part of August each year. Data obtained in 1927 were again in agreement with those of 1928 and are not shown. The material was dried at 100° C. for a period of about one-half hour, followed at 60° C. until an approximately constant weight was reached. Analyses were made the following winter in each case. The dried samples were ground to pass a no. 80 mesh screen. The unmodified Kjeldahl method was used for determination of nitrogen, as little nitrate nitrogen has been reported in apple wood. Official methods of the Association of Official Agricultural Chemists were used in making carbohydrate determinations.

ANATOMICAL STRUCTURE.—Transverse sections 15–30 μ thick were made with the sliding microtome from samples fixed and stored as previously described. These were stained with safranin and aniline blue or with safranin and Delafield's haematoxylin and mounted in balsam. Camera lucida diagrams of cross sections of pith tissue were made from material prepared in this manner.

HIGH AND LOW NITROGEN NUTRIENT.—Yearling trees of several varieties propagated on dwarf stocks were grown in the greenhouse in 14-inch pots containing practically pure quartz sand from January, 1928, to May, 1928, and then transferred to out-of-doors for the remainder of the season. Stock nutrient solutions prepared according to the following formula were used:

WITH NITROGEN		WITHOUT NITROGEN	
A		A	
KNO_3	40 gm.	$MgSO_4$	20 gm.
$MgSO_4$	20 "	K_2HPO_4	20 "
$Ca(H_2PO_4)_2$	20 "	KH_2PO_4	10 "
Water	920 cc.	Water	950 cc.
B		B	
$Ca(NO_3)_2$	60 gm.	$CaSO_4$	Saturate
Water	940 cc.	Water	1000 cc.

The nutrient solution as supplied to the potted trees was prepared by diluting A and B each with seven parts of water and mixing together. One-half liter of this nutrient was added to each pot at about 10-day intervals during the period of most active growth, and less often later in the season. Six to eight trees of each variety were given the nutrient containing nitrogen and an equal number given the nutrient without nitrogen. All trees were watered with tap water which contained traces of nitrogen. The records describing the growth made by the trees during 1928 (table III) were taken in October. The trees were then removed from the pots and stored in the cellar until January, 1929, when they were again potted and placed in the greenhouse but no nutrient other than that contained in the tap water was supplied.

Definitions

The terms fruitful shoot and fruitful growth have reference to the growth produced when fruit buds are formed and not when fruit is actually borne. (The blossoms appearing on a tree in May were differentiated the previous summer.) The terms non-fruitful shoot and unfruitful growth refer to the growth produced when no fruit buds are formed. Thus, on biennially bearing trees, a fruitful growth is made the season when the tree is not bearing fruit, and an unfruitful growth when the tree is bearing fruit.

In the discussion of the anatomical structure of the xylem, the terms spring wood and summer wood are used. These terms designate a quality of the xylem rather than a time of formation. The first formed inner xylem, in which vessels are large and abundant, is spoken of as spring wood although not always formed in the spring. The last formed xylem, in which vessels are few and small and in which parenchymatous and fibrous cells are abundant, is spoken of as summer wood or summer xylem.

Presentation of data

Character of terminal shoot growth.—The quality of the terminal shoots as to length, diameter, degree of tapering, length of internodes, brashness, and size of terminal leaves is thought to be indicative of the physiological condition of the tree.

The character of the terminal shoot growth produced by trees of representative varieties and fruiting types is shown in figure 1. The fruitful terminal growths made by trees of biennially bearing varieties were found to taper but little from base to tip, terminal internodes were long, and the last leaves formed were large, the wood itself being brashy. The unfruitful terminal shoot growth made by trees of biennially bearing varieties was willowy and tapered markedly from base to tip, terminal internodes were short, and last formed leaves were small. The terminal shoot growth made

by trees of regularly bearing varieties was observed to be moderately brashy and moderately thick from base to tip, terminal internodes were moderately long, and last leaves formed were moderately large. The terminal shoot growth made by trees of shyly bearing varieties was somewhat willowy and slender, terminal internodes tended to be short, and last formed leaves moderately small.

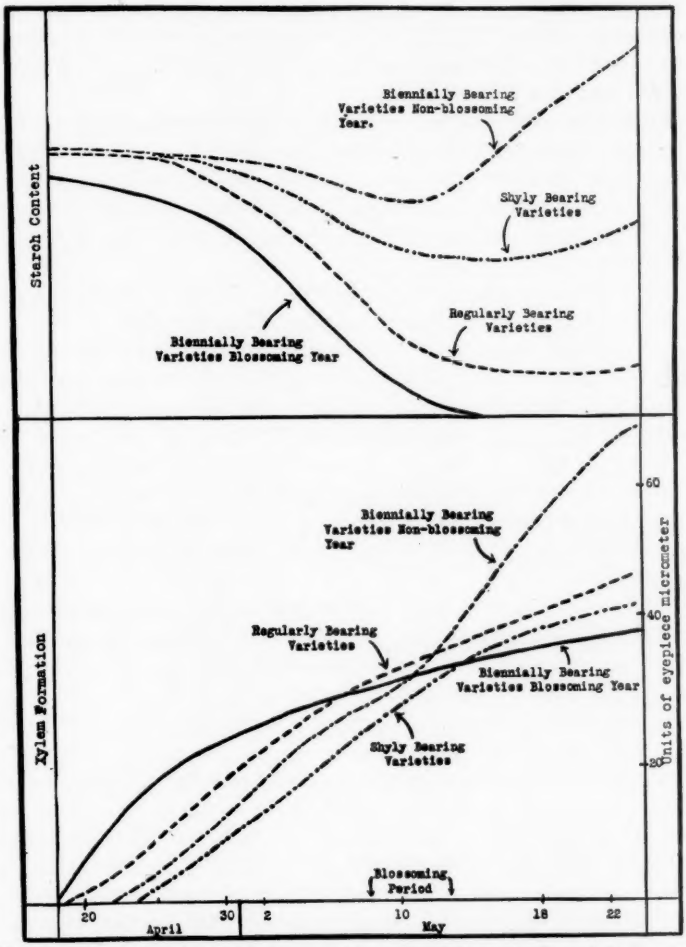

FIG. 2. Formation (width) of new xylem and relative starch content near tips of previous season's growth. Estimated from microscopic observations, spring of 1928, Madison, Wisconsin.

This description of growth character applies only when trees are fruiting typically for the variety. When a tree of a variety of one class fruited like trees of another class of varieties, the character of the terminal shoots produced was found to be correlated to and typical of the condition of fruitfulness.

STARCH DISAPPEARANCE.—The rate and completeness with which starch and cell wall thickenings (reserves) disappeared from the previous season's shoots prior to, during, and following blossoming were studied as an indication of the relative carbohydrate utilization in the shoots of the different classes and varieties of trees.

The disappearance of starch from the previous season's shoots was first initiated near growing points, as apical meristems and blossom buds, in biennially bearing trees which were entering the blossoming year (fig. 2). This disappearance was followed in point of time by regularly bearing trees, shyly bearing trees, and biennially bearing trees which were entering the non-blossoming year.

In the blossoming year of the biennially bearing trees starch disappearance continued until after blossoming, when little remained in any of the tissues of the small branches. A large part of the reserve present as cell wall thickenings in the pith also disappeared, particularly in regions close to blossom buds, apparently being utilized in fruiting and in growth.

In the non-blossoming year of the biennially bearing trees, starch disappeared but little from the phloem, cortex, and xylem; a considerable amount remained in the pith. Starch which disappeared with the starting of growth in the spring was soon replaced and the content remained relatively high throughout the summer. Few if any of the cell wall thickenings of the pith disappeared.

In the regularly bearing and in the shyly bearing trees starch was depleted less rapidly and less completely than in the blossoming and fruiting year of biennially bearing trees, but more rapidly and more completely than in the non-fruiting year of biennially bearing trees.

RATE AND PERIOD OF TERMINAL GROWTH.—As has been suggested by many workers, the rate and period of terminal growth of a shoot may be influenced by the composition or nutritional condition of the shoot; hence shoots differing with respect to nutrition should have a different rate and period of terminal growth. With this thought in mind, the rate and period of elongation of the terminal shoots of trees of different varieties and classes of growth and fruiting were measured. The data for 1928 only are shown (fig. 3), being alike for the two years observed. All shoots of the class of which each curve is representative had growth curves markedly uniform as to configuration, although the total length of the shoots measured was found to vary within a class.

The rate and period of terminal growth of the unfruitful terminal shoots of biennially bearing trees were found to be markedly different from that of the very fruitful growth of biennially bearing trees. The unfruitful shoot started growing later, its growth proceeded slowly during the early part of the growth period, it put on smaller leaves, and ceased elongation abruptly. The fruitful growth started much earlier and more rapidly, put on large leaves, and ceased elongation gradually. The growth of the

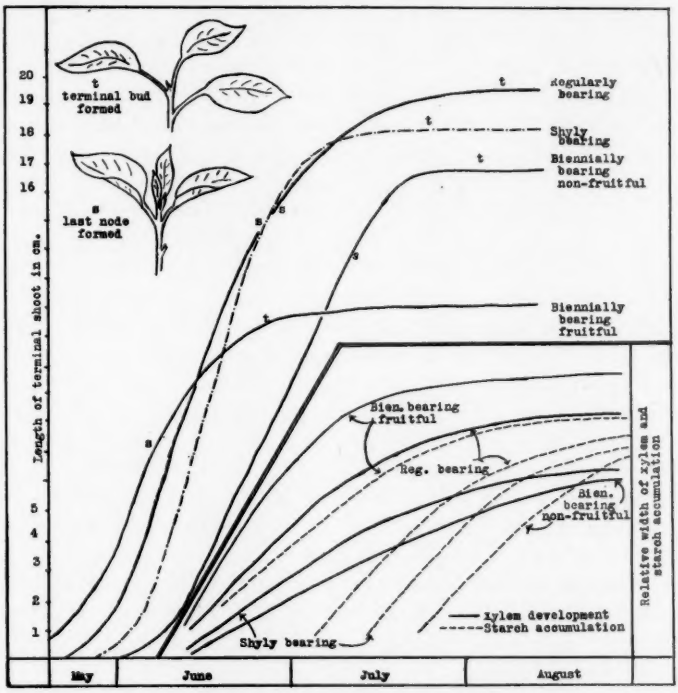

FIG. 3. Curves of terminal shoot growth, xylem development, and estimated starch accumulation in terminal shoots (third internode from tip) of trees of the various growth and fruiting classes. Sturgeon Bay, 1928.

terminal shoots of the regularly bearing trees was intermediate in respect to time of starting growth, initial rate, and abruptness with which growth ceased. This was particularly true when equal lengths of all types of shoots were compared. Long terminal shoots of regularly bearing trees started growing in the spring nearly as fast as shorter but more fruitful growths of biennially bearing trees. The leveling of the growth curve of the terminal shoots of the shyly bearing varieties (Spy and Liveland) suggested

that of the curves for the unfruitful growths of biennially bearing trees.

The relative amount of terminal elongation of the shoots after the last node has been formed (fig. 3, *S*) is of interest and possibly of significance. This elongation is due to lengthening of internodes. It may be that the short terminal internodes of unfruitful shoots and the abrupt flattening of their growth curve are related to a common fundamental condition, and likewise the long terminal internodes and gradual flattening of the growth curve of the fruitful shoots.

If the form of the growth curve is influenced by the internal nutrition or composition of the shoot, then one interpretation that may be made of the elongation curves of figure 3 is that terminal shoots of trees of the different varieties and classes are unlike as to composition. The curves of the fruitful and of the unfruitful terminal shoots of biennially bearing trees possibly represent extremes of composition, those of shoots of regularly and shyly bearing trees indicating an intermediate composition.

XYLEM FORMATION.—A correlation of diameter thickness to fruiting performance has been suggested for spurs by CROW and EIDT (**1**) and for spurs and terminal shoots by ROBERTS (**5**).

The formation of secondary xylem in the current season's terminal shoots of trees of the different varieties and classes was found to be related to fruitfulness; that is, to blossom bud formation, in the shoots (fig. 3). It was initiated first, and proceeded most rapidly in the fruitful terminal shoots of biennially bearing trees. It was next initiated and proceeded moderately rapidly in the terminal shoots of regularly bearing trees. It was initiated later and proceeded slowly in the terminal shoots of shyly bearing trees. It was initiated last and proceeded most slowly in the unfruitful terminal shoots of biennially bearing trees.

The widest xylem (measured at a point usually in the third internode below the tip of the shoot) was in the fruitful terminal shoots of biennially bearing trees. The terminal shoots of regularly and shyly bearing trees had the next widest xylem, and the unfruitful terminal shoots of biennially bearing trees had the narrowest xylem (figs. 4, 5). The xylem at the base of the shoot may be wider in a long growth such as a sucker or the terminal shoot of a regularly bearing tree than in the fruitful shoot. The width of the xylem in these cases, however, appears to be associated with the elongating of the shoot. The quality of such basal xylem, as indicated by anatomical structure, was not like that in the fruitful shoot. Spring xylem development in one-year old wood follows the initiation of starch disappearance and the initiation of terminal shoot growth. It is correlated with these and not directly with blossom bud formation.

STARCH ACCUMULATION.—Starch accumulation in the terminal shoots was investigated as a possible index of the carbohydrate composition of the

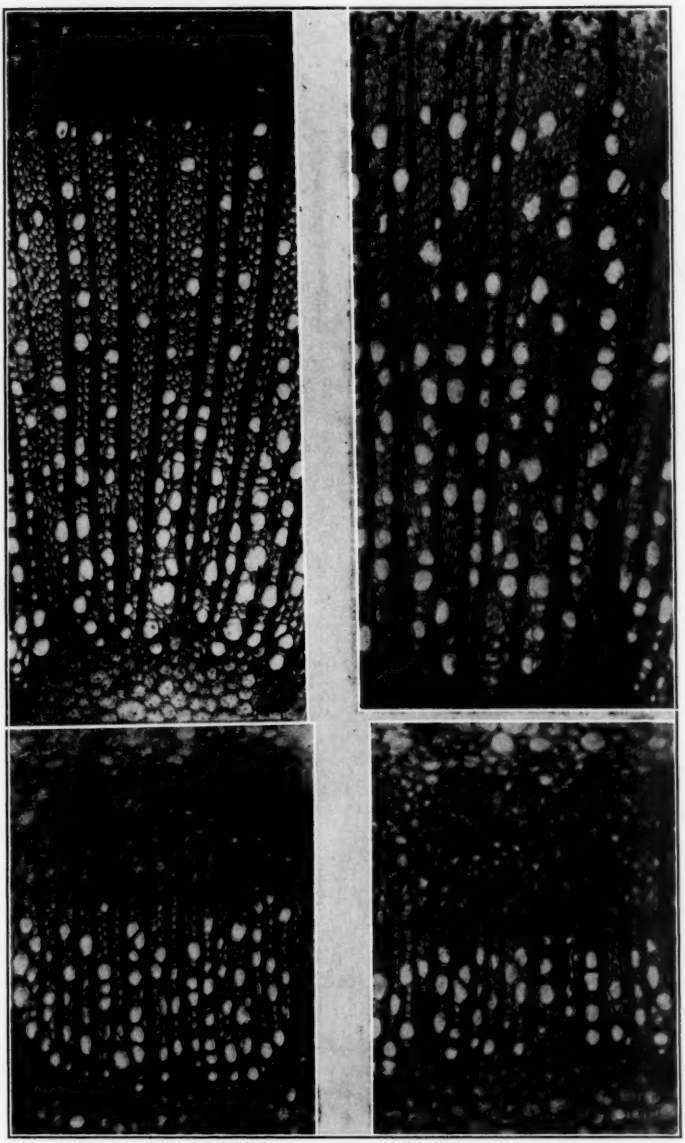

FIG. 4. The xylem in the fruitful growths of biennially bearing trees is much wider than that in the unfruitful growths. It has an abundance of summer wood containing many parenchymatous cells.

A. Wealthy fruitful.
B. Duchess fruitful.
C. Wealthy unfruitful.
D. Duchess unfruitful.

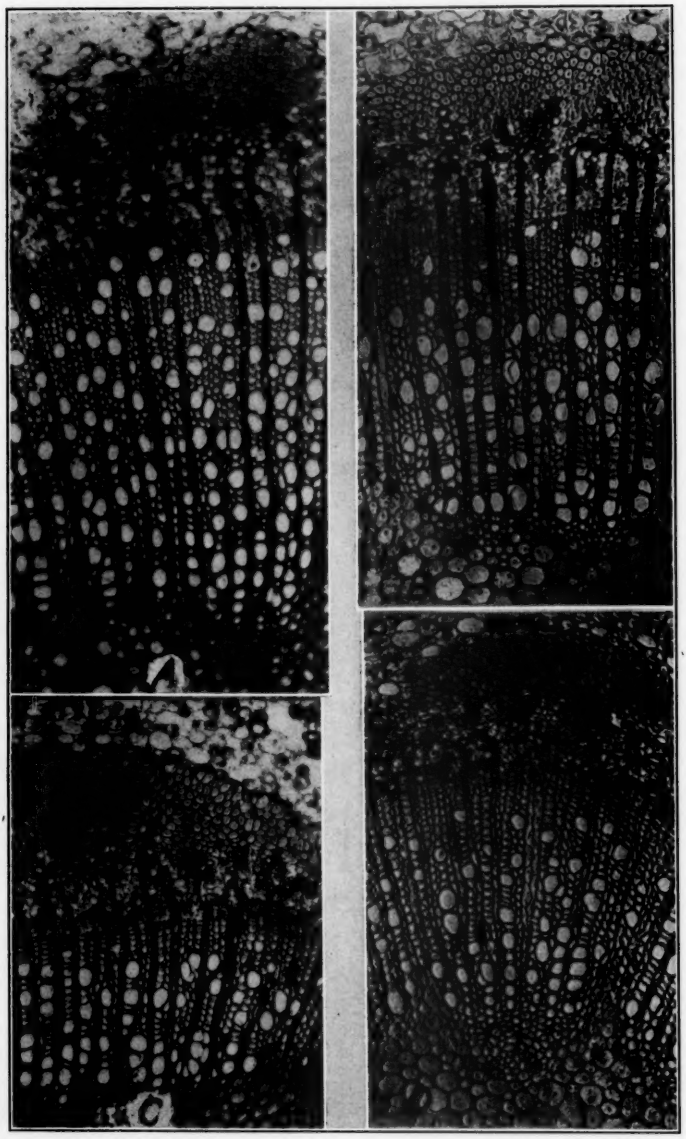

FIG. 5. The xylem in the terminal shoots of regularly and shyly bearing trees is intermediate in width and amount of summer wood but that of the shyly bearing approaches the non-fruitful extreme.

A. Northwestern. B. McIntosh.
C. Newell. D. Spy.

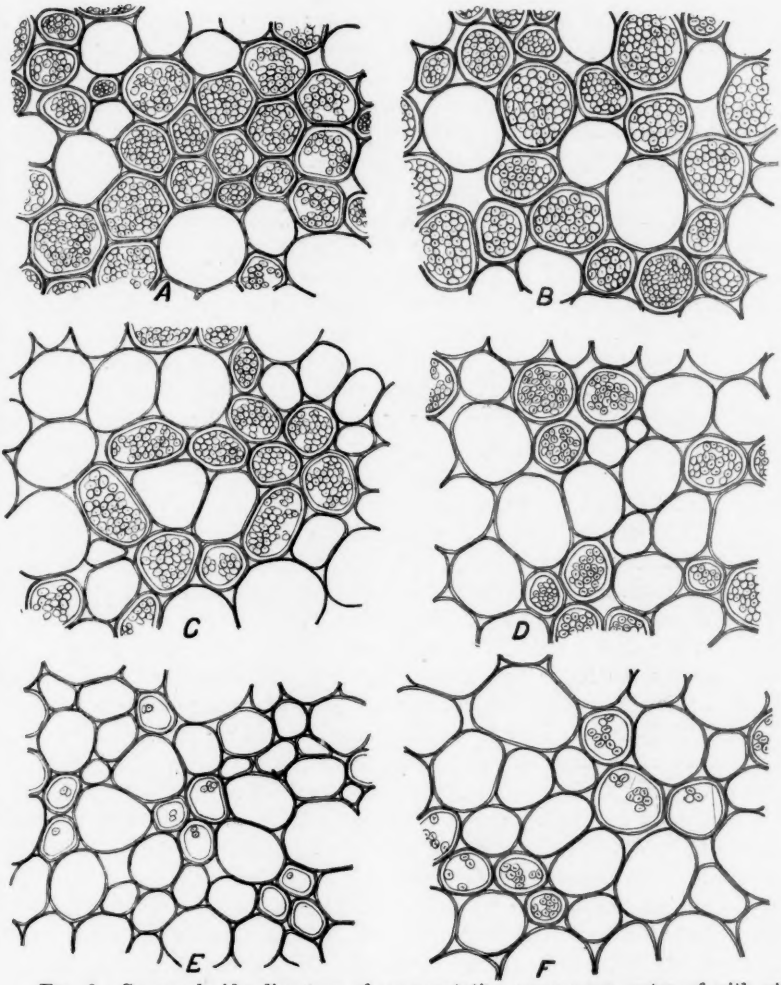

FIG. 6. Camera lucida diagrams of representative areas near center of pith at base of nursery whips.

A. Northwestern girdled.
B. Wealthy untreated.
C. Northwestern untreated.
D. Fameuse untreated.
E. Spy untreated.
F. Northwestern defoliated.

different shoots and as one factor associated with their fruiting performance. In the current season's terminal shoots, starch accumulation begins at the base and proceeds upward. This accumulation and the formation of the

xylem progress uniformly in the current season's growth. In older wood the presence of lateral branches and spurs causes local accumulations of starch and irregularities in width of xylem.

Starch appeared first in the storage tissues of the fruitful terminal shoots of biennially bearing trees, and last in the unfruitful shoots of biennially bearing trees. The appearance of starch in the terminal shoots of typically bearing trees of regularly and shyly bearing varieties occurred after that in the fruitful shoots but preceded that in the unfruitful shoots of the biennially bearing trees (fig. 3).

The formation of the secondary thickenings of the pith cell walls appeared to precede the formation of starch in the cell. Starch was not found in pith cells which did not have secondary thickenings (fig. 6). Inversely it was observed that starch is removed from pith cells in the spring before the wall thickenings disappear. The total width of the thickenings was observed to vary between the different types of shoots, as does also the relative number of cells in the pith having such wall thickenings.

A considerable amount of starch was present in the fruitful growths by June 20 (1928), whereas a comparable amount had not accumulated in unfruitful terminal shoots until the middle of July. About the first of August starch began to accumulate abundantly in all shoots. By the early part of September, it appeared that nearly as much starch was present in the unfruitful as in the fruitful growths, yet blossom buds were formed only on the fruitful shoots where starch storage took place early in the season. By the latter part of September or early October starch began disappearing from the terminal shoots. This disappearance was observed to be initiated at the base of the shoot and proceed upward.

Chemical composition of terminal shoots.—Data obtained from macrochemical analyses of tissue collected in 1928 appear in table I. The values found for percentage of ether extractable materials, free reducing and total reducing substances, do not show a consistent relation to growth character or to the amount of blossom bud formation in the shoot. The acid hydrolyzable fraction was somewhat correlated with the degree of fruitfulness of the shoots of different varieties. Of the samples analyzed, starch content was found to be highest in the fruitful shoots of biennially bearing trees and lowest in the unfruitful shoots. The starch-nitrogen and total carbohydrate-nitrogen ratios were greatest in the fruitful and lowest in the unfruitful shoots. Evidently typically fruiting trees of biennially bearing varieties build up carbohydrates abundantly in relation to nitrogenous compounds the season that many fruit buds are formed. The next season, when the fruit is borne and no blossom buds are formed, they are relatively low in carbohydrates. Trees of regularly and shyly bearing varieties formed an intermediate number of fruit buds, and produced a terminal

TABLE I

COMPOSITION OF TERMINAL SHOOTS COLLECTED JULY 27, 1928, STURGEON BAY, WISCONSIN

	DUCHESS UNFRUITFUL	WEALTHY UNFRUITFUL	NORTHERN SPY	NORTH-WESTERN GREENING	McINTOSH	DUCHESS FRUITFUL	WEALTHY FRUITFUL
Percentage ether extract	2.98	3.32	2.38	2.41	2.20	2.38	2.36
Percentage free reducing substances	2.96	3.07	2.18	2.67	2.31	2.57	2.59
Percentage total reducing substances	4.87	5.45	3.14	5.23	4.50	4.62	4.33
Percentage starch	2.41	2.57	3.91	2.86	2.95	4.86	5.95
Percentage acid hydrolyzable substances	5.03	5.41	5.27	5.54	6.77	6.27	6.09
Percentage total carbohydrates	12.31	13.43	12.32	13.63	14.22	15.75	16.37
Percentage total nitrogen	0.930	0.868	0.759	0.830	0.894	0.792	0.777
Ratio $\frac{\text{starch}}{\text{nitrogen}}$	2.59	2.80	5.15	3.44	3.30	6.13	7.65
Ratio $\frac{\text{total carbohydrates}}{\text{nitrogen}}$	13.20	15.5	16.20	16.40	16.00	19.80	21.1

growth that was intermediate in carbohydrate and nitrogen composition between that produced during the "on" and "off" years of biennial kinds.

Shoots selected at random from those collected for macro-analyses were sectioned and observed microscopically. The results of these studies were in general agreement with the analytical data, although the former indicate wider extremes of starch and hemicellulose content than were revealed in the macro-analyses.

These data cannot be interpreted as meaning that a maximum carbohydrate content makes for fruitfulness, since samples were taken only from the "fruitful range." An unfruitful high carbohydrate-low nitrogen sample (**3**) was not included.

The numerical values assigned to the ratios in table I are relative and not absolute. An absolute expression of chemical composition does not seem feasible. This is partly because of the failure of present methods of macro-analyses accurately to measure materials used in growth and fruiting. As in the present data, the ordinary methods of analyses recover only a part of the total material in the sample, any metabolic value that the remainder may have not being estimated. Again there is the question of whether data showing composition should be expressed as percentage or in amounts per given unit. Anatomical studies have indicated that fruiting is associated with a quality of composition rather than with a quantity of materials. If such is true the percentage basis may provide a more accurate index by which the composition as related to fruiting may be expressed.

ANATOMICAL STRUCTURE.—That the anatomy of the plant may reflect its vegetative and fruiting condition has been suggested by numerous workers, yet little use has been made of this method as one approach to the solution of certain physiological problems. In the present study consideration of anatomical features provided additional information.

The xylem was found to vary widely in the different classes of shoots as to amount and quality (numbers and kinds of cells, size of cells, etc.) of summer wood present. The xylem of the fruitful growths of biennially bearing trees had the most summer wood; that of the unfruitful terminal growths had the least (fig. 4). Intermediate between these two extremes was the amount of summer wood present in the xylem of the terminal shoots of regularly and shyly bearing trees (fig. 5).

The summer wood characteristic of the fruitful terminal shoot of a biennially bearing tree was found to have fewer vessels than the spring wood of the same shoot, or of the spring wood comprising practically the entire xylem in the unfruitful shoot. Other differences were also found which may have much significance from the standpoint of the chemical composition of the shoot, and particularly of changes in composition throughout the period of xylem formation. Outstanding among these were

the numbers and ratios of ray cells, vessels, fibers, and parenchyma (table II and figs. 4 and 5).

TABLE II

RATIOS OF FIBERS, RAY CELLS, AND VESSELS TO PARENCHYMA IN OUTER XYLEM OF SHOOTS OF VARIOUS VARIETIES GROWING UNDER SEVERAL CONDITIONS

	FIBERS: PARENCHYMA	RAY CELLS: PARENCHYMA	VESSELS: PARENCHYMA
TERMINAL SHOOTS OF ORCHARD TREES			
Wealthy unfruitful	5.8	.....	.....
Duchess unfruitful	5.9	.....	.....
Spy	5.5	.....	.....
Newell	5.4	.....	.....
Northwestern	5.2	1.8	0.74
McIntosh	4.6	1.7	0.75
Fameuse	3.3	1.3	0.40
Wealthy fruitful	2.4	0.72	0.19
Duchess fruitful	2.5	0.74	0.20
Wealthy, under-vegetative and unfruitful	2.0	0.80	0.20
BASE OF NURSERY WHIPS			
Spy	11.6	1.7	0.58
Fameuse	6.0	0.86	0.44
Wealthy	4.6	0.65	0.36
Northwestern, defoliated	12.9	1.8	0.65
Northwestern, untreated	6.2	0.98	0.30
Northwestern, girdled	3.8	0.76	0.35
POTTED WEALTHY TREES			
Grown in shade with high nitrogen nutrient, girdled, sample taken below girdle	13.3	7.4	1.9
Grown in full sunlight with low nitrogen nutrient, girdled, sample from above girdle	2.5	1.4	0.45

The summer xylem of the fruitful terminal shoots of biennially bearing trees had a relatively large number of parenchymatous cells, and the fiber to parenchyma ratio was low. The summer xylem of the terminal shoots of regularly bearing trees had a smaller number of parenchymatous cells, and the fiber to parenchyma ratio was higher. The summer xylem, of which there was very little in the terminal shoots of shyly bearing trees and in the unfruitful shoots of biennially bearing trees, contained few parenchyma cells and relatively many fibers. The same relationship of cells in the spring wood did not appear to have this definite trend from the fruitful to the unfruitful shoots. Spring wood varied less throughout the different types

of shoots than did the summer wood. The quality of the early formed spring wood appeared to be related to the presence and utilization of food reserves. Summer wood seemed related to the current supply and accumulation of elaborated foods during the growth period.

That the character of the anatomical structure of the xylem may be markedly influenced by the chemical composition of the shoot was indicated by a study of the anatomy in Wealthy trees which represented wide extremes of growth character, fruitfulness, and composition (table II).

1. Shoots on which no blossom buds formed were obtained from an old, poorly vegetative, unfruitful, nitrogen starved tree. The xylem of such shoots was composed almost entirely of summer wood in which parenchyma was abundant and the ratio of fibers to parenchyma was the lowest of any samples examined. It has been reported (**5**) that samples similar to these have a high carbohydrate and low nitrogen composition.
2. Shoots on which many blossom buds formed, and which corresponded in external appearance to the fruitful terminal shoots of biennially bearing trees, were obtained from trees growing in pot culture. In these the xylem contained considerable summer wood in which the number of parenchyma cells was relatively high and the ratio of fibers to parenchyma was relatively low. It has been reported (**5, 6**) that such shoots have a relatively high carbohydrate and intermediate nitrogen content.
3. Shoots on which no blossom buds formed were taken from below a girdle on shaded trees growing in pots with a high nitrogen nutrient. The xylem of such shoots contained little summer wood and in it the number of parenchyma cells was small and the ratio of fibers to parenchyma was high. It has been reported (**6**) that samples similar to these have a low carbohydrate and high nitrogen content.

If the chemical composition of these types of shoots has been correctly reported, is it not further evidence that:

1. The fruitful terminal shoots of biennially bearing varieties (which have in the xylem a large amount of summer wood containing much parenchyma and few fibers) are of a relatively high carbohydrate and low nitrogen composition;
2. The terminal shoots of trees of regularly and shyly bearing varieties (which have in the xylem somewhat less summer wood and parenchyma and more fibers) are of somewhat lower carbohydrate and higher nitrogen composition;
3. The unfruitful terminal shoots of trees of biennially bearing varieties (which have in the xylem little summer wood, few parenchyma,

and many fibers) are of relatively low carbohydrate and high nitrogen composition?

A study of the anatomy of nursery whips also yielded further evidence. Some were defoliated and others girdled during midsummer. Sections of these and of untreated trees taken in late fall after growth had ceased showed that the relative amount of parenchyma in the last formed xylem was least in defoliated, next highest in untreated, and highest in girdled trees (table II). The relative carbohydrate content as observed in the pith of these trees is represented in the camera lucida diagrams of figure 6. The carbohydrate-nitrogen ratio has been found by HARVEY (**2**) to be least in defoliated, next highest in untreated, and highest in girdled shoots.

Nursery whips of different varieties were found to differ in number of parenchymatous cells and in the parenchyma to fiber ratio in the outer xylem. Of Spy, Fameuse, and Wealthy, Spy had relatively the lowest number of parenchyma cells, comparable to defoliated Northwestern. Fameuse was comparable to Northwestern untreated; Wealthy had relatively the greatest number of parenchymatous cells and was comparable to girdled Northwestern (table II). Of these same nursery trees the amount of starch and the secondary cell wall thickenings in the pith were greatest in Northwestern girdled and Wealthy untreated; were intermediate in Northwestern and Fameuse untreated; were least in Northwestern defoliated and Spy untreated (fig. 6).

If the relative number of parenchyma cells in the xylem and the relative amount of starch and secondary cell wall thickenings of the pith give an index of the amount of carbohydrates present in young trees, then the Wealthy, even in the nursery row, starts to accumulate carbohydrates fairly abundantly, Fameuse and Northwestern less so, and Spy least of all.

It may be significant that the age at which trees of these varieties ordinarily come into bearing is in the same order as the rate or degree to which these data indicate carbohydrates to be formed in the young nursery trees. The growth made by young trees of all varieties indicates that they tend to be of a higher nitrogen composition than older trees. As carbohydrates accumulate the tree makes less and less growth, a condition of fruitfulness finally resulting. Trees of those varieties which form carbohydrates most abundantly usually reach this condition first, depending upon cultural conditions. Similarly, when grown with a deficiency of nitrogen, they are often the first to reach, later in life, an unfruitful condition in which the character of growth produced is characteristic of an excessively high carbohydrate composition.

RESPONSE TO HIGH AND LOW NITROGEN NUTRIENT.—If trees of different varieties differ in formation and use of carbohydrate and nitrogen compounds, then the amount and quality of the growth produced by trees of

TABLE III

GROWTH OF ONE-YEAR OLD DWARF TREES IN POTS WITH HIGH AND LOW NITROGEN NUTRIENT. GROWN IN THE GREENHOUSE FROM JANUARY 30 TO MAY 14 AND THEN OUT-OF-DOORS UNTIL OCTOBER

	HIGH NITROGEN					LOW NITROGEN				
	WEALTHY	DUCHESS	MC-INTOSH	NORTH-WESTERN	SPY	WEALTHY	DUCHESS	MC-INTOSH	NORTH-WESTERN	SPY
Total growth (cm.)	75	48	137	175	83	29	24	58	55	77
Increase in weight (per cent.)	411	495	360	286	298	112	189	153	149	305
Fruitful	Yes	Yes	No	No	No	No	No	No	No	No

different varieties when grown under similar and controlled nitrogen nutrient conditions might be different.

It was found that potted trees of different varieties did not respond alike to similar nutrient conditions (table III). Under high nitrogen nutrient, Duchess and Wealthy trees were the only ones to form blossom buds the first season after potting. Trees of the other varieties were over-vegetative; that is, a relatively long, tapering, unfruitful growth was made. With low nitrogen nutrient conditions, Duchess and Wealthy trees were under-vegetative; that is, a short slender growth was made; the growth of McIntosh closely approached the fruitful character but no fruit buds were formed. Northwestern and Spy were still over-vegetative in growth type.

It is significant that under a high nitrogen nutrient Spy showed almost the least and Wealthy the greatest relative increase in weight; under low nitrogen nutrient Spy showed the greatest and Wealthy the least increase (table III). Spy increased in weight more in low nitrogen nutrient than in high; Wealthy and Duchess gained more weight in high nitrogen than in low.

In January of 1929 these trees were again potted, placed in the greenhouse, and allowed to grow without soil nutrient other than that contained in tap water. The amount of growth made under these conditions would seem fairly indicative of the quality or amount of previous seasons' reserves present in the trees. Of the trees previously grown in high nitrogen nutrient, Duchess and Wealthy made the most growth (total length), McIntosh and Northwestern less, and Spy least. This small amount of growth of Spy was apparently because of a deficiency of carbohydrate reserves formed in 1928. Of the trees which had been in low nitrogen nutrient in 1928, Spy made the most growth, Duchess and Wealthy least. This small amount of growth of Duchess and Wealthy was apparently because of a deficiency of nitrogen reserves formed in 1928.

Discussion

The recognition that the cause of the difference in fruiting performance between varieties may be related to differences in growth character as induced by differences in carbohydrate and nitrogen utilization by the trees provides a basis for planning cultural treatments. Growth type and carbohydrate and nitrogen composition can be altered in the orchard easily, practically, and in many instances economically, by such ordinary practices as pruning, the application of readily available nitrogenous fertilizers, and by other soil management programs. Since some varieties tend to be relatively higher in nitrogen and some relatively higher in carbohydrates, the same amount or kind of pruning, the same fertilizer applications, or the same soil management may have opposite effects when applied to different

varieties. The adaptation of cultural treatments to a variety must be based on the performance of that variety under the conditions and locality in which it is being grown. These can be only suggested here.

Trees of such varieties as Spy, Newell, and Golden Russett, upon reaching a bearing size, should be treated to increase the carbohydrate content in order to increase fruitfulness. Less of a nitrogenous fertilizer need be applied. Pruning should be of the thinning-out type to allow more sunlight to reach the leaves. Bending of branches to admit sunlight may be a practical means of inducing early bearing. This has been reported (**4**) to have increased the yields of 9-year-old Spy trees in New York.

By comparison, trees of such varieties as Duchess, Wealthy, or Transparent should be treated to avoid an excessively high carbohydrate content. This can be done by soil management practices which increase the available nitrogen of the soil; and by pruning, particularly of the heading-back type which gives the response of a lowered carbohydrate content or of an increased nitrogen content.

The present study suggests further evidence for the view that the fruiting performance of a tree is dependent upon the nutritional condition existing within it, rather than directly upon any specific environmental influence. It has emphasized the importance, as has been set forth by other workers, of a condition of balance between extremes in growth character and chemical composition of the trees as favoring fruitfulness. While genetic differences are perhaps the underlying causes for the differences in varietal behavior, they are expressed in orderly and more or less understandable physiological processes, a knowledge of which furnishes a basis for cultural treatment.

An interesting as well as an important fact that has come from this study is the relation between chemical composition, fruitfulness, growth character, growth rate and period, rate and period of xylem formation, presence or absence of summer wood, and cell distribution in the xylem. It may be that information can be obtained of one phase of plant performance by considering it in relation to others.

A study of the anatomical structure of the plant has been used as one means of measuring its physiological condition. This method of attack may prove of further value in the solution of some physiological problems, particularly in view of the present uncertainty of macrochemical methods to yield completely satisfactory results and also in view of the relative ease with which some seasonal nutritional changes can be detected anatomically.

The present study has emphasized the importance of the time and initial rate of growth in determining the fruitfulness of the resulting shoot. Further knowledge is needed of the chemistry of food reserves upon which growth in the spring is largely dependent.

Summary

1. The data presented give evidence that the relative position between the extremes of vegetativeness that trees of a variety normally occupy is correlated with the typical fruiting performance of trees of the variety. The degree of vegetativeness is closely related to or determined by the chemical composition of the tree, particularly as regards carbohydrate and nitrogen contents.

2. Of the samples analyzed, the starch and total carbohydrate contents and starch-nitrogen and total carbohydrate-nitrogen ratios were highest in fruitful terminal shoots of trees of biennially bearing varieties. They were next highest in terminal shoots of trees of regularly and shyly bearing varieties. They were lowest in the unfruitful terminal shoots of trees of biennially bearing varieties. (Nitrogen starved non-fruitful trees were not analyzed.)

3. Numerical expressions of chemical composition, particularly of carbohydrate-nitrogen ratios, are only relative because of limitations of present methods of macro-analyses.

4. Terminal growth was first initiated and proceeded more rapidly during the early part of the growing period in the fruitful than in the unfruitful terminal shoots of trees of biennially bearing varieties. Terminal growth of trees of regularly and shyly bearing varieties was intermediate in point of time of initiation and initial rate.

5. Accumulation of starch and formation of xylem were initiated earlier and proceeded more rapidly in the fruitful terminal shoots of trees of biennially bearing varieties than in the unfruitful terminal shoots. These were intermediate in the terminal shoots of trees of regularly and shyly bearing varieties.

6. The width of xylem in the terminal shoots of trees of regularly and shyly bearing varieties was intermediate between that of unfruitful and fruitful terminal shoots of trees of biennially bearing varieties.

7. Summer wood formation in the xylem was associated with carbohydrate accumulation. It was abundant in the xylem of fruitful terminal shoots of trees of biennially bearing varieties. It was less abundant in terminal shoots of trees of regularly and shyly bearing varieties and least abundant in unfruitful terminal shoots of trees of biennially bearing varieties.

8. A relatively large number of parenchymatous cells and a low fiber to parenchyma ratio in the xylem were associated with a high carbohydrate, low nitrogen composition. Conversely, a relatively small number of parenchymatous cells and a high fiber to parenchyma ratio in the xylem were associated with a low carbohydrate, high nitrogen composition.

9. Trees of different varieties propagated on dwarf stocks did not respond equally to similar nutrient conditions in amount and character of growth produced.

10. Fruitfulness was correlated with character of terminal growth, growth rate and period, rate and period of xylem formation, presence of summer wood, cell distribution in the xylem, and chemical composition.

11. The time and rate at which terminal growth, with its accompanying leaf formation, are initiated in the spring appeared to be highly correlated with the fruitfulness of the resulting growth.

12. The chemical composition of trees of a variety as indicated by the character of the terminal shoot growth may be used as an index to cultural needs.

13. The unfruitful growths of biennially bearing trees represent an extreme of composition. The fruitful growths represent an intermediate condition. A possible other extreme of samples from nitrogen-starved trees was not included.

Gratitude is extended to Dr. R. H. ROBERTS, who suggested this study and gave counsel throughout the course of the work, and to Dr. W. E. TOTTINGHAM and others of the plant chemistry department, University of Wisconsin, who gave helpful advice and kindly permitted the use of their laboratory for conducting the macrochemical analyses.

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DETERMINATION OF THE NITROGENOUS FRACTIONS IN VEGETATIVE TISSUE OF THE PEACH[1]

O. W. DAVIDSON AND J. W. SHIVE

Introduction

All vegetative parts of the peach contain a glucoside which may be hydrolyzed to form hydrocyanic acid, benzaldehyde, and glucose by the action of any enzyme, or enzymes, also present in the various tissues. The cyanogenetic glucoside or glucosides are not present in the same concentration in all parts of the plant. Thus in the fruit flesh it is found only in very minute amounts, although some investigators (**12**) have reported it to be absent from this part. Cyanogen compounds are known to occur in many plants, however, but they are supposed to be present only as glucosides. Nevertheless, WILLAMAN (**26**) and others have found in some plants what was regarded as either free hydrocyanic acid or hydrocyanic acid from a very unstable glucoside. Although no free hydrocyanic acid is known to exist in the peach, the cyanogenetic glucoside, or glucosides, are either more stable in dormant or slowly growing plants than in rapidly growing ones, or else their specific enzymes are relatively inactive in the slowly growing plants.

Because of the presence of a nitrogenous glucoside, or glucosides, peach tissue cannot be macerated in the preparation of an extract of soluble nitrogen without a consequent loss of nitrogen. Certain modifications in the procedure commonly employed for plant analyses are necessary, therefore, and the nitrogen contained in this glucoside must be determined and removed completely from an aqueous extract of peach tissues before the remaining nitrogenous fractions can be determined accurately.

In the present study an attempt has been made to modify the methods commonly employed for the determination of the nitrogenous fractions in plants in general, so that they may be used for investigations of these fractions in the tissues of the peach (*Prunus persica* Stokes).

The methods described are suitable for use with samples comprising 50 gm. or more of fresh plant material. Samples of this size have been found adequate to give representative results. Only aqueous extracts prepared from fresh plant material were regarded as suitable for use in this connection, since CHIBNALL (**4**) has shown that such extracts are more applicable than any others in a study of the nitrogen distribution in plants.

The investigation is admittedly incomplete in that it does not include a study of the composition of all parts of the peach plant sampled at various

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

stages of growth. There may be parts of the plant that at times will require special handling previous to extraction, such as that indicated by ROBINSON (**14**).

Materials and methods

In these studies the stem material used consisted of twigs one year old or less taken from peach trees grown in an orchard or in sand cultures in a greenhouse. Only the non-woody portions of the root systems of peach trees grown in sand cultures were analyzed. The material consisted of the tender white ends of roots from which the cortex had not sloughed off, and 3–5 cm. of the older portions from which the cortex was missing.

PREPARATION OF TISSUE FOR EXTRACTION

In the preparation of an extract from peach tissue, it is necessary that the enzyme or enzymes which hydrolyze cyanogenetic glucosides be destroyed as early in the process as possible. Unless the material is handled quickly and precautions are taken to prevent or minimize the hydrolysis of the glucoside, some of the hydrocyanic acid liberated may be lost during the preparation of the extract. Before the enzyme is destroyed, however, the material must be prepared for aliquoting and extracting.

Accordingly the twigs were ground to shreds in a pencil sharpener (**7**). When entirely woody, the whole twig could be ground in this manner. In the case of actively growing shoots, however, the terminal 8–10 cm. of growth, and the short, slender side branches could not be ground satisfactorily in a pencil sharpener because of lack of rigidity in the tissue. Such material was therefore minced finely with pruning shears and then mixed thoroughly with the shredded material and aliquoted.

METHOD OF EXTRACTING

In studying the nitrogen distribution in the leaves of *Prunus laurocerasus,* ROBINSON (**14**) placed a small sample of minced leaves in a flask, added cold water, and then inserted a stopper fitted with tubes to permit a strong current of air to be passed through the contents. The aeration was started and the flask was heated until the contents reached boiling point, at which it was held for 20 minutes. Any hydrocyanic acid distilled over was collected in 5 per cent. sodium hydroxide contained in a flask immersed in an ice bath. By continuing the aeration for three hours after boiling, she presumably recovered any hydrocyanic acid liberated from the tissue before the emulsin was destroyed. Through this procedure she recovered half or more of the cyanide present in cherry laurel leaves. The remaining cyanogenetic nitrogen was recovered by the action of emulsin upon an aliquot of the aqueous extract.

Inasmuch as *P. laurocerasus* and *P. persica* are closely related species, it was expected that peach stem tissue subjected to this treatment would likewise yield a portion of the nitrogen contained in its cyanogenetic glucoside. The amounts of hydrocyanic acid recovered from samples of peach tissue boiled and aerated in this manner[2] varied widely. In the case of dormant and slowly growing peach stems no hydrocyanic acid was liberated. Samples comprised of the terminal 15–20 cm. of stems that were growing rather slowly in late August, however, liberated a very small amount of cyanogenetic nitrogen equivalent to only 0.0022 per cent. of their green weight. At the same time the stem tips of fast growing suckers taken from young trees yielded cyanogenetic nitrogen equivalent to 0.0145 per cent. of their green weights. During June, when the peach trees were growing most rapidly, a sample comprised of the terminal 15–20 cm. of rapidly growing stems liberated cyanogenetic nitrogen equivalent to as much as 0.0438 per cent. of the green weight. This sample contained a relatively large proportion of meristematic tissue rich in nitrogenous constituents. Nevertheless the unusually high proportion of hydrocyanic acid recovered in the distillate indicates that the cyanogenetic glucoside found in rapidly growing peach stems is less stable than that found in slowly growing stems, or else that the emulsin present in the former is more active than that in the latter.

It is interesting to note that no hydrocyanic acid was liberated when the leaves of rapidly growing peach stems were boiled and aspirated. The short period of time required for the preparation of a sample of the leaves before bringing them to a boil may have been too brief to allow an appreciable amount of hydrolysis of the cyanogenetic glucoside to take place.

VIEHOEVER, JOHNS, and ALSBERG (**22**) used various treatments for distilling the cyanogenetic nitrogen from *Tridens flavus*. Their results showed that no hydrocyanic acid, or at the most only a very small proportion, was liberated when the tissue was distilled without the addition of acid.

It is evident that a wide range of stability is exhibited by the various cyanogenetic glucosides found in plants. Indeed there is considerable indication of a variation in the stability of the cyanogenetic glucoside or glucosides found within a single plant (**26**).

The preliminary boiling under a reflux condenser recommended by ROBINSON was therefore omitted in the preparation of extracts from dormant and slowly growing peach stems, and from roots and leaves. In this case an aqueous extract was prepared by covering a 50-gm. aliquot of the finely minced plant material with boiling water in a large beaker, and

[2] In this work, hot instead of cold water was added to the samples in the distilling flasks in order to hasten the destruction of enzymes and to minimize the liberation of hydrocyanic acid from the tissue.

allowing the contents to boil for 20 minutes. Following this the tissue was transferred to "longcloth" and washed repeatedly, then placed in a mortar where the pieces minced with shears were pounded to a pulp. A further extraction was accomplished by boiling the tissue again for 10 minutes and then washing it several times, to make a volume of approximately 950 cc. This method is essentially the same as that adopted by VICKERY and PUCHER (**21**) for an investigation of the nitrogenous fractions in tobacco leaves. It has been studied recently by DAVIDSON, CLARK, and SHIVE (**6**) for use with a number of plants, including the peach.

When actively growing peach stems were extracted, it was necessary to use the preliminary distillation and aspiration recommended by ROBINSON. For this purpose the sample was placed in a 2-liter Florence flask and covered with 400 to 500 cc. of boiling water. After the aspiration had been continued for three hours, the sample was extracted as described in the preceding paragraph.

Owing to the bulkiness of the shredded peach stem tissue, samples heavier than 50 gm. were seldom used, and samples weighing from 80 to 100 gm. were always extracted with about 1900 cc. of water in order to insure a thorough removal of the soluble nitrogen present. Since minced peach roots are much less bulky than minced or shredded peach stems, root samples heavier than 50 gm. could be extracted conveniently with 1 liter of water.

A peach extract obtained in this manner contains very little nitrogen that can be coagulated by heating with very dilute acetic acid. Nevertheless the extract, having a volume of approximately 900 to 950 cc., was heated to boiling in a large beaker, 1.5 cc. of 10 per cent. acetic acid was added, and the solution was allowed to boil for 1 minute. The hot extract then was filtered through paper pulp in a Buchner funnel with the aid of suction.

In a previous investigation (**6**), the amounts and distribution of soluble nitrogen in representative aliquots of peach stems were compared after extraction with boiling water as just described, and after grinding in a mortar with the aid of sand, followed by extraction with cold water. By the latter method the glucoside probably was exposed to the active enzyme for more than an hour longer than it was by the boiling method. During this period of maceration in the mortar, 11.66 per cent. of the cyanogenetic nitrogen present either was lost or was combined in some stable compound that did not permit its release in the subsequent treatments. ALSBERG and BLACK (**1**), working with *Prunus virginiana* and *Andropogon sorghum*, recovered less hydrocyanic acid after a period of maceration than by the distillation of fresh comminuted leaves.

CYANOGENETIC NITROGEN

An analysis of the various nitrogenous fractions in an extract of peach tissue presents little difficulty after the complete removal of cyanogenetic

nitrogen has been effected. Indeed for the analysis of an extract free of this form of nitrogen, the methods employed by NIGHTINGALE *et al.* (**9, 10**), TIEDJENS and ROBBINS (**19**), TIEDJENS and BLAKE (**20**), and others (**5**) may be used with only slight modifications.

The quantitative recovery of cyanogenetic nitrogen in peach tissue extracts, however, has presented considerable difficulty. A review of the literature dealing with methods for determining this form of nitrogen in plants has revealed a variety of procedures, none of which is entirely satisfactory.

DETERMINATION BY ACID HYDROLYSIS.—The hydrolysis of amygdalin by acids and by emulsin has attracted the attention of chemists for many years. AULD (**2**), WALKER and KRIEBLE (**25**), and later KRIEBLE (**8**), have made special studies of the methods and products of the hydrolysis of amygdalin. These investigations have shown that the nitrogen in amygdalin is converted readily to ammonium sulphate by the action of concentrated sulphuric acid. When treated with dilute sulphuric acid, however, the hydrolysis takes a different course, forming l-mandelonitrile, from which hydrocyanic acid is liberated slowly and incompletely. These studies have shown clearly that the hydrolysis of amygdalin, with the subsequent liberation of hydrocyanic acid, is accomplished more rapidly and more completely by the action of emulsin than by that of acids.

ALSBERG and BLACK (**1**) concluded that all of the hydrocyanic acid that could be obtained from the leaves of *Prunus virginiana* was liberated during hydrolysis by boiling with 5 per cent. sulphuric acid for 4 hours, whereas that recovered from *Andropogon sorghum* and *Panicularia nervata* was liberated during hydrolysis for 1 hour with the same concentration of acid. This conclusion, however, was based upon the assumption that all of the cyanogenetic nitrogen present in the glucosides was liberated when further acid hydrolysis failed to release any more hydrocyanic acid, an assumption that has been shown (**26**) to be unjustified.

It was considered here that information concerning the extent to which hydrocyanic acid is liberated from extracts of peach tissue during hydrolysis with sulphuric acid might be of considerable importance. Thus if it were found that this form of nitrogen could be removed quantitatively by such treatment, then, by subtraction, Kjeldahl determinations before and after hydrolysis would reveal the amount of cyanogenetic nitrogen present. Furthermore, if this fraction could be removed during the 2.5-hour hydrolysis with 5 per cent. sulphuric acid used in the determination of amide nitrogen, the analysis would be simplified by the elimination of one procedure.

Accordingly aliquots of an extract prepared from peach stems were hydrolyzed by boiling for different lengths of time under condensers of

the Hopkins type with two concentrations of sulphuric acid. The amounts of hydrocyanic acid liberated were determined by a comparison of the amounts of total nitrogen in the aliquots before and after hydrolysis. Similar aliquots of the same extract also were hydrolyzed with emulsin, following a method that will be described later. The results obtained, together with data showing the behavior of c.p. amygdalin when hydrolyzed with dilute sulphuric acid and with emulsin, are presented in table I. It is apparent that, during acid hydrolysis, the behavior of the cyanogenetic glucoside in a peach stem extract is very similar to that of amygdalin. It is also apparent that the decomposition of the glucoside by hydrolysis with dilute sulphuric acid is far from complete. Nevertheless duplicate aliquots, after hydrolysis for the same time and with the same concentration of acid, always yielded similar amounts of nitrogen. This fact would seem to indicate that, under similar conditions of hydrolysis, the decomposition of the glucoside stops at a fairly definite stage.

TABLE I

COMPARISON OF AMOUNTS OF HYDROCYANIC ACID NITROGEN LIBERATED FROM ALIQUOTS OF A PEACH STEM EXTRACT AND FROM A 1% SOLUTION OF AMYGDALIN BY HYDROLYSIS WITH DILUTE SULPHURIC ACID AND WITH EMULSIN

TREATMENT	TOTAL NITROGEN	YIELD OF HCN–N	RECOVERY OR LOSS OF HCN–N
	gm.	*gm.*	%
100 cc. of stem extract, Kjeldahlized	0.00860		
100 cc. of stem extract, hydrolyzed with emulsin	0.00555*	0.00309	100†
100 cc. of stem extract, hydrolyzed 2.5 hours with 5% H_2SO_4	0.00649	0.00211	69
100 cc. of stem extract, hydrolyzed 5 hours with 5% H_2SO_4	0.00607	0.00253	82
100 cc. of stem extract, hydrolyzed 5 hours with 10% H_2SO_4	0.00656	0.00203	66
100 cc. of 1% amygdalin, Kjeldahlized	0.02813		
100 cc. of 1% amygdalin, hydrolyzed with emulsin		0.02810	100
100 cc. of 1% amygdalin, hydrolyzed 2.5 hours with 5% H_2SO_4	0.00778	0.02055	73

* The amount HCN–N recovered by hydrolysis with emulsin is taken as 100% of that present in the aliquot, although the actual recovery from c.p. amygdalin was 99.89%.

† This figure does not include any of the nitrogen added by emulsin.

DETERMINATION BY ENZYMATIC HYDROLYSIS.—WILLAMAN (**26**), after obtaining unusually low yields of hydrocyanic acid from amygdalin by

means of hydrolysis with 5 per cent. sulphuric acid, abandoned this procedure for the analysis of plant tissues. He therefore chose emulsin as an hydrolytic agent and recovered as much as 98.5 per cent. of the theoretical amount of cyanide nitrogen present in samples of amygdalin when the enzyme was allowed to operate over a period of 24 hours.

In the more recent work of ROBINSON (**14**), the cyanogenetic glucosides in *Prunus laurocerasus,* as well as in *Andropogon sorghum,* were hydrolyzed by emulsin and the hydrocyanic acid formed was removed by aspiration for 3 hours and collected in 5 per cent. sodium hydroxide. This method has the advantage of being much more rapid than that used by WILLAMAN, since the removal of hydrocyanic acid favors a more complete hydrolysis of the glucoside. This may be anticipated from the work of AULD (**2**), who has shown that the hydrolysis of amygdalin by emulsin may not go to completion in the presence of the hydrolytic products. KRIEBLE (**8**) has shown also that emulsin has synthetic properties in the presence of hydrocyanic acid and benzaldehyde.

It is of interest to note that none of the investigators studying cyanogenesis in plants recognized any need for adjusting the solution containing the glucoside to a reaction favorable to the hydrolytic activity of emulsin. Although this enzyme is active over a fairly wide range of pH values, its optimum range is comparatively narrow. VULQUIN (**23**) found that regardless of the agents used to adjust the reaction of the solution, and regardless of the quality of the emulsin used, the maximum cleavage of amygdalin occurs when the hydrogen ion concentration is between pH 5.2 and 5.7. WILLSTÄTTER and CSANYI (**27**) concluded that a reaction between pH 5.0 and 6.5 is favorable to the hydrolysis of amygdalin by emulsin, and that the optimum reaction is approximately pH 6.

Inasmuch as the reaction of extracts of peach tissue prepared as described previously was usually about pH 4.6 to 4.7, it was considered advisable to determine the reaction at which such extracts yield the maximum amounts of hydrocyanic acid when hydrolyzed with emulsin. For this purpose a 1-liter extract was prepared from 50 gm. of peach stems (var. Cumberland), and duplicate 100-cc. aliquots of this were adjusted to the reactions shown in table II. The amounts of cyanogenetic nitrogen present were then determined by the method recommended by ROE (**15**). Thus 0.05[3] gm. of emulsin was added to each aliquot contained in a 300-cc. Florence flask, which was then closed by inserting a rubber stopper containing aspiration tubes sealed by clamps placed on the rubber connections.

[3] In later analyses, 0.02 gm. of emulsin was found adequate for such extracts. Since some preparations of emulsin have a high content of nitrogen, a known amount of the enzyme should always be added and an excess avoided when the extract is to be partitioned for the determination of other nitrogen fractions.

The aliquots were warmed to 40°–50° C. for 15 to 30 minutes, after which each flask was connected to a correspondingly numbered flask containing 100 cc. of 5 per cent. sodium hydroxide. The flasks were arranged in a series and a strong current of air was drawn through them for 4 hours. The cyanide collected in the sodium hydroxide flasks was titrated with N/100 silver nitrate, using 10 drops of 10 per cent. potassium iodide as an indicator. At the end of the period of aspiration the reactions of the various aliquots were determined again and were found to have changed in all but one instance. This change was due probably to the fact that the initial pH readings were taken before equilibrium in the extract had been established.

The amounts of cyanogenetic nitrogen obtained from the various aliquots during this experiment are shown in table II. The maximum recovery, that at pH 5.7, is expressed as 100 per cent. for comparison with the recovery at other reactions. That the amount recovered at pH 5.7 may be very close to 100 per cent. of the cyanogenetic nitrogen present in the extract is indicated by the fact, as shown in table I, that 99.89 per cent. of the nitrogen present in aliquots of a 1 per cent. solution of amygdalin adjusted to this reaction was recovered under similar conditions.

TABLE II

COMPARISON OF EFFECTS OF HYDROGEN ION CONCENTRATION OF EXTRACT UPON AMOUNTS OF CYANOGENETIC NITROGEN RECOVERED FROM PEACH STEMS

INITIAL pH	FINAL pH	CYANOGENETIC NITROGEN RECOVERED	
		GRAMS PER 50 GM. OF TISSUE	PERCENTAGE RECOVERY
		gm.	%
5.0	5.1	0.02381	95.9
5.0	5.0	0.02381	95.9
5.5	5.3	0.02410	97.1
5.5	5.3	0.02410	97.1
6.0	5.7	0.02497	100.0
6.0	5.7	0.02468	
6.5	6.0	0.02410	97.1
6.5	6.1	0.02381	95.9
7.0	6.6	0.02265	87.2
7.0	6.5	0.02322	93.5

Another experiment similar to the one just described was carried out using a 1-liter extract prepared from 50 gm. of stem tissue taken from a different tree (*P. kansuensis* × *P. persica* hybrid). One hundred cc. aliquots of this extract were adjusted to reactions ranging from pH 4.0 to 7.0, as shown in table III. After standing for 4 hours the reactions showed a change of from 0.1 to 0.5 of a pH unit. The solutions were readjusted to the desired reactions, therefore, 0.05 gm. of emulsin added to each, and the

cyanogenetic nitrogen determined as in the preceding experiment. Subsequent changes in the reactions of the solutions were very slight. The amounts of cyanogenetic nitrogen found are presented in table III, where the maximum recovery is expressed as 100 per cent. for comparative purposes.

TABLE III

COMPARISON OF EFFECTS OF HYDROGEN ION CONCENTRATION OF EXTRACT UPON AMOUNTS OF CYANOGENETIC NITROGEN RECOVERED FROM PEACH STEMS

pH	Cyanogenetic nitrogen recovered	
	Grams per 50 gm. of tissue	Percentage recovery
	gm.	%
4.0	0.02621	63.8
4.5	0.03717	90.5
5.0	0.03891	94.7
5.5	0.04109	100.0
6.0	0.04029	98.1
6.5	0.03731	90.8
7.0	0.01713	41.7

From these two experiments it is apparent that the optimum reaction for the enzymatic hydrolysis of the cyanogenetic glucosides in an extract of peach tissue is approximately pH 5.5 to 5.7. It is apparent also that a reaction between pH 5.5 and 6.0 is satisfactory for most analytical purposes. This range of hydrogen ion concentrations is in good agreement with those found by Vulquin (**23**) and by Willstätter and Csanyi (**27**) for enzymatic hydrolysis of amygdalin.

In this connection it is of interest to note that the reaction of an extract of peach stems prepared without the addition of any acid was found to be approximately pH 5.3. Such a reaction, of course, is too low for the maximum recovery of the cyanogenetic nitrogen present. This does not mean, however, that the emulsin naturally present in peach tissues functions at pH 5.3. Cells in the phloem of the peach range in reaction from pH 5.4 to approximately pH 6.5, whereas most of the cells in the xylem have reactions ranging from pH 4.2 to 4.4. The extract of peach stems just described represents a composite sample of the soluble constituents of all tissues present, and its reaction should therefore be intermediate between the highest and the lowest reactions found in the cells.

It is apparent, therefore, that for the complete recovery of the cyanogenetic nitrogen from extracts of peach tissue, as well as from solutions of amygdalin, an enzymatic hydrolysis should be employed. It is evident also that this hydrolysis should be conducted at a reaction between pH 5.5 and 6.0, and that the hydrocyanic acid formed should be removed rapidly.

Ammonium nitrogen

Robinson (**14**) displaced ammonia from extracts of *P. laurocerasus* with a saturated solution of borax during distillation *in vacuo* at 60° C., after the method of Watchorn and Holmes (**24**). Since it has been shown (**16, 19**) that when 0.5 per cent. sodium hydroxide was substituted for a saturated solution of sodium carbonate in the method of Sessions and Shive (**16**), slightly but consistently larger amounts of ammonia were recovered from plant extracts, it was anticipated that the use of borax would not displace all of the ammonium nitrogen present in peach extracts. Tiedjens (**18**) found that the amount of ammonium nitrogen recovered by aspiration with 0.5 per cent. sodium hydroxide corresponded to the amount recovered by electrodialysis.

Aliquots of a peach stem extract were analyzed for ammonium nitrogen, both by the method of Watchorn and Holmes and by the method of Sessions and Shive, using 0.65 per cent. sodium hydroxide.[4]

When the results of the two methods were compared (table IV), the difference in the amounts of ammonium nitrogen recovered was far greater than was expected. It was obvious that some form of nitrogen, not normally present as ammonium, was being liberated by the treatment with dilute sodium hydroxide but not by the treatment with borax.

TABLE IV

Comparison of amounts of ammonium nitrogen recovered from peach stem extracts in the presence of cyanogenetic nitrogen and ferrous sulphate

Treatment	Nitrogen recovered from 50 gm. of tissue
	gm.
1. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen	0.0363
2. Aspirated with 0.65% NaOH after removal of cyanogenetic nitrogen	0.0064
3. Distilled *in vacuo* with a saturated solution of borax in presence of cyanogenetic nitrogen	0.0024
4. Distilled *in vacuo* with a saturated solution of borax after removal of cyanogenetic nitrogen	0.0023
5. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen plus 5 mg. of $FeSO_4 \cdot 7H_2O$	0.0200
6. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen plus 500 mg. of $FeSO_4 \cdot 7H_2O$	0.0076

[4] In preliminary work it was found that 0.65 per cent. sodium hydroxide displaced very consistent amounts of ammonia from peach extracts, whereas the use of 0.5 per cent. sodium hydroxide did not always give good agreement among duplicates.

In order to obtain further information regarding this point, the aliquots used were made neutral to litmus, adjusted to a reaction between pH 5.5 and 6.0, and the amount of cyanogenetic nitrogen present determined. No cyanogenetic nitrogen was liberated after the treatment with dilute sodium hydroxide, and only 68 per cent. of the amount previously found to be present was recovered from aliquots treated with borax. Moreover, the data shown in table V indicate that the increase in the amount of nitrogen recovered during aspiration with 0.65 per cent. sodium hydroxide does not account for all of the cyanogenetic nitrogen present in the extract. The results show also that both of the methods used for the determination of ammonium nitrogen in the presence of cyanogenetic nitrogen either caused a loss of the same amount of the latter, or caused the same amount of the glucoside to be affected in such a manner that it was stable toward emulsin.

As a means of studying the behavior of the cyanogenetic glucoside at room temperatures, solutions of amygdalin were aspirated with different concentrations of alkali and were found to liberate varying amounts of the nitrogen present. The results showed that the nitrogen in amygdalin may be wholly or partially converted to ammonia during hydrolysis with 0.65 per cent. sodium hydroxide at room temperatures. It was noticed, however, that the first one or two flasks in a series (those nearest to the suction pump) always liberated more ammonia than did any of the others. The

TABLE V

SUMMARY OF FACTORS AFFECTING RECOVERY OF CYANOGENETIC NITROGEN FROM PEACH STEM EXTRACTS

TREATMENT	GRAMS IN 50 GM. OF STEMS
	gm.
1. Cyanogenetic nitrogen recovered from initial aliquots by hydrolysis with emulsin	0.0482
2. Cyanogenetic nitrogen recovered by aspiration with 0.65% NaOH	0.0299
3. Cyanogenetic nitrogen recovered after hydrolysis of (2) by emulsin	0.0000
4. Cyanogenetic nitrogen tied up during aspiration with 0.65% NaOH	0.0153
5. Cyanogenetic nitrogen recovered by hydrolysis with emulsin following displacement of ammonia by borax	0.0330
6. Cyanogenetic nitrogen tied up during displacement of ammonia by borax	0.0152
7. Nitrogen in addition to that from amides recovered after hydrolysis of (3) with 5% H_2SO_4 for 2.5 hours (emulsin blank deducted)	0.0159

erratic results thus obtained with amygdalin were not typical of the behavior of peach extracts during similar treatment.

The failure to obtain complete recovery of cyanogenetic nitrogen following ammonia determinations suggested that ferro- or ferricyanides might be formed by a reaction between the soluble iron compounds present and any cyanide liberated from the glucoside in an alkaline solution. The nitrogen involved in such a reaction would of course be combined in an undissociated complex, from which it could be recovered neither by alkaline nor by enzymatic hydrolysis. The presence of such compounds in peach extracts, however, could not be established definitely. Nevertheless, the addition of ferrous sulphate to an extract before aspiration with 0.65 per cent. sodium hydroxide always resulted in the recovery of less nitrogen than was obtained by similar treatment without the addition of the iron salt. In table IV it will be observed that, when 500 mg. of ferrous sulphate were added to an extract, the amount of nitrogen recovered corresponded closely with, but was somewhat higher than, the amount of ammonium nitrogen found in the extract, indicating that practically all of the cyanogenetic nitrogen was combined in some way with the iron salt.

Referring to table V again, it is observed that the cyanogenetic nitrogen not recovered from a peach extract during aspiration with 0.65 per cent. sodium hydroxide was recovered quantitatively after hydrolysis by boiling for 2.5 hours with 5 per cent. sulphuric acid. This demonstrated that no cyanogenetic nitrogen was lost during ammonia determinations, and showed that it was not combined as an iron cyanide complex, since the latter during hydrolysis with dilute sulphuric acid should form hydrocyanic acid instead of an ammonium salt.

It is apparent, therefore, that ammonium nitrogen in peach extracts should be determined by aspiration with sodium hydroxide only after the removal of cyanogenetic nitrogen. Also, since the use of 0.65 per cent. sodium hydroxide permits a more nearly complete displacement of ammonium nitrogen from the extracts, it is preferable to either borax or sodium carbonate for this purpose, especially since the addition of these substances produces a high salt concentration which often interferes with aliquoting for amino nitrogen.

Total soluble nitrogen

Following the removal of cyanogenetic and ammonium nitrogen, the aliquots in which these fractions were determined were neutralized, made faintly acid by the addition of a drop of acetic acid, and then evaporated to dryness. The amount of nitrogen remaining in the aliquots was estimated by Kjeldahlization as modified to include nitrates. The sum of cyanogenetic, ammonium, and Kjeldahl values gave the amount of total soluble nitrogen present.

Amide nitrogen

It was shown in table I that, when a peach extract was hydrolyzed with 5 per cent. sulphuric acid for 2.5 hours, the procedure usually employed in the determination of amides, only about 75 per cent. of the cyanogenetic nitrogen present was liberated as hydrocyanic acid. In table V it was shown that, when the cyanogenetic nitrogen was not removed completely before the amide hydrolysis, the values obtained for the latter fraction were high. It is apparent, therefore, that the cyanogenetic nitrogen should be removed before amide nitrogen is determined.

Since emulsin contains a high percentage of nitrogen, it was realized that either this substance must be removed after the hydrolysis of the glucoside, or the amounts of nitrogen added by it to the amide, basic, amino, total soluble, etc., fractions must be determined. When, however, attempts were made to precipitate emulsin and to filter it from extracts, its complete removal was never satisfactorily accomplished. Robinson (**14**) likewise reported inability to remove by precipitation the nitrogen added by emulsin.

In order to determine how the addition of emulsin to a peach extract would affect the various nitrogenous fractions studied, duplicate 0.0500-gm. samples of the enzyme were subjected to precisely the same procedure as were those used in the analysis of the extracts. The results presented in table VI show that the amounts of emulsin ordinarily added to an extract increase appreciably only the total soluble, amide, total amino, and basic nitrogen fractions. Hence it is obvious that, when the nitrogenous composition of the emulsin preparation is known and when carefully weighed amounts of the latter are used, the nitrogen thus added to the above fractions of the extract may be deducted.

During the present study the extent to which the nitrogenous composition of different preparations of emulsin may vary was not investigated,

TABLE VI

Distribution of nitrogen in 0.0500 gm. samples of emulsin used in hydrolysis

Fraction	Amount of nitrogen found	
	gm.	%
Total N	0.00559	11.180
Cyanogenetic N	None	None
Ammonium N	0.00006	0.012
Amide N	0.00099	0.980
Humin N	None	None
Total amino N	0.00112	2.232
Mono amino N	0.00008	0.016
Basic N	0.00168	3.360

since material from only a single preparation was used. Nevertheless it may be anticipated from the studies of WILLSTÄTTER and OPPENHEIMER (**28**) that different preparations of emulsin might vary in their composition.

The following method was therefore adopted for the estimation of amide nitrogen in peach extracts: After the removal of two 100-cc. aliquots used for cyanogenetic, ammonium, and total soluble nitrogen determinations, the remaining 800 cc. of a 1-liter extract were evaporated to 200 cc. and divided into two equal portions. Since the remaining soluble nitrogen fractions in the extract were present only in very small amounts, their estimation in concentrated aliquots avoided the use of an unnecessarily large aliquot factor. Cyanogenetic nitrogen was then removed from each of these aliquots as described previously. Sulphuric acid sufficient to give a concentration of 5 per cent. was added and the amide nitrogen present was hydrolyzed to ammonium nitrogen by boiling under condensers for 2.5 hours. The aliquots were then neutralized and the total ammonium nitrogen present determined by aspiration with 0.65 per cent. sodium hydroxide. The increase in nitrogen recovered over that found previously in the ammonia was amide nitrogen.

HUMIN NITROGEN

Following the removal of amide nitrogen, the aliquots were neutralized and the acid insoluble humin precipitate formed during the amide hydrolysis was filtered off. The precipitate was washed thoroughly with hot distilled water, dried, and Kjeldahlized. The nitrogen found in this precipitate is referred to as humin nitrogen.

NITRATE NITROGEN

Nitrate nitrogen was determined by the method of SESSIONS and SHIVE (**16**) on aliquots free of cyanogenetic nitrogen. Since 0.65 per cent. sodium hydroxide was used for the reduction with Devarda's alloy and for the subsequent displacement of ammonia, cyanogenetic nitrogen, if present, would be converted partially to ammonia and would therefore give high values for the nitrate fraction.

Accordingly this fraction was sometimes estimated in the same aliquots in which the ammonium nitrogen had been determined, but the presence of Devarda's alloy often made it inconvenient to use these samples for the Kjeldahlization of the remaining soluble nitrogen. It was found very convenient, however, to estimate nitrates in 25 cc. of the filtrate from the humin precipitate after it had been made to a volume of 100 cc.

BASIC NITROGEN

The phosphotungstic acid method of OSBORNE and HARRIS (**11**) was employed to precipitate the basic nitrogen present in 50-cc. aliquots of the

filtrate from the humin precipitate. The basic precipitate was not washed with a dilute solution of the precipitating reagent, since it was found that such treatment often dissolved some of the precipitate and caused poor agreement between duplicates. The aliquots were filtered through small sized filter paper and the precipitates allowed to drain overnight. Inasmuch as the filtrate contained only a very low percentage of nitrogen, the amount of non-basic nitrogen retained in the precipitate by this method of handling was slight.

Amino nitrogen

Amino nitrogen was always estimated by the Van Slyke method on 10-cc. portions of the filtrate from the humin precipitate, and on similar portions of the filtrate from the basic precipitate. With the exception of extracts prepared from young, actively growing trees, the amounts of amino nitrogen found in peach extracts of the concentrations used in this study were very small. Because of this, a gas burette from a micro-Van Slyke apparatus was fitted on a macro-apparatus in order to permit the more accurate reading of the gas evolved.

Total nitrogen

It was anticipated from the work of Ranker (**13**) that the Kjeldahl method, modified to include nitrates, would not recover all of the nitrogen present when determinations were made on green tissue containing nitrates. Strowd (**17**) has shown also that appreciable amounts of nitrates are recovered by Kjeldahl determinations even without the addition of reducing agents. Hence it seemed inadvisable to use the ordinary Kjeldahl method without the modification to include nitrates, and then to add to the amount of nitrogen recovered any nitrate nitrogen found by separate determinations to be present. It is evident that, for the accurate recovery of total nitrogen in the presence of nitrates, only dried plant material should be used; nevertheless the work of Robinson (**14**) indicates that a loss of nitrogen as hydrocyanic acid may take place during the drying of peach tissue.

In accordance with these considerations, different lots of peach tissue, some containing nitrates and some free of nitrates, were analyzed for total nitrogen by the use of a sulphuric-salicylic acid mixture and zinc to fix and reduce nitrates. Samples consisting of 8 gm. of green tissue were weighed rapidly but carefully in triplicate, and covered immediately with the sulphuric-salicylic acid mixture. Other aliquots of the same tissues were placed in an oven operated at 103° to 105° C. for one-half hour, after which the temperature was lowered to 65°–70° C. until the material was dry. These dried samples were ground in a drug mill, heated to 70° C. for one hour, and then cooled in a desiccator. Total nitrogen determinations were run on 1-gm. portions of the dried material, using the same procedure as

that used on the green tissue. A comparison of the results is shown in table VII.

TABLE VII

COMPARISON OF QUANTITIES OF TOTAL NITROGEN, EXPRESSED AS PERCENTAGE OF DRY WEIGHT, RECOVERED BY KJELDAHL DETERMINATIONS ON GREEN AND DRY TISSUE, WITH AND WITHOUT THE PRESENCE OF NITRATES

TISSUES USED	PECENTAGE OF NITRATE-NITROGEN PRESENT IN DRY TISSUE	NITROGEN RECOVERED FROM GREEN TISSUE	NITROGEN RECOVERED FROM DRY TISSUE
	%	%	%
Stems	0.000	1.695	1.718
Stems	0.000	1.498	1.524
Stems	0.000	1.260	1.267
Stems	0.041	1.182	1.281
Roots	0.000	4.026	3.931
Roots	0.023	3.750	3.694
Roots	0.944	3.311	3.675
Roots	2.518	3.439	3.974

It is obvious that peach tissue loses little if any of its nitrogen during the drying process. There is some indication of a slight loss of nitrogen from root tissue while drying, but there is no evidence of any loss occurring from stem tissue during similar treatment. The tender, white, non-woody portions of the peach roots used in these analyses contained considerably more of the cyanogenetic glucoside on a dry weight basis than did the stems. At the same time it is possible that the glucoside contained in peach roots is less stable than that found in the stems. WILLAMAN (**26**) believes that sorghum does not contain all of its cyanogenetic nitrogen in the form of dhurrin, and that at times a part, or all, of this form of nitrogen may exist in some less stable compound.

Peach roots may also contain a high percentage of nitrates, all of which may not be recovered by the modified Kjeldahl determination, unless the tissue is thoroughly dry before the sulphuric-salicylic acid mixture is added, and unless the digestion is conducted very slowly.

DISTRIBUTION OF NITROGEN

The amounts and distribution of nitrogen in peach trees vary tremendously with the conditions under which they are grown and with the activity of the plants at the time of sampling. In order to illustrate these variations, the percentages of the different nitrogenous fractions found in seedlings of Early Crawford, sampled while still growing in a greenhouse on November 1, are compared in table VIII with similar fractions found in the variety Cumberland sampled in the orchard on March 16. Although

TABLE VIII

NITROGENOUS FRACTIONS IN PEACH STEMS AND ROOTS EXPRESSED AS PERCENTAGE OF GREEN AND DRY MATTER, AND AS PERCENTAGE OF TOTAL NITROGEN

	STEMS FROM DORMANT TREES IN ORCHARD 3/16/33			ROOTS* FROM GROWING TREES IN GREENHOUSE 11/1/32			STEMS* FROM GROWING TREES IN GREENHOUSE 11/1/32		
	GREEN	DRY	TOTAL N AS 100%	GREEN	DRY	TOTAL N AS 100%	GREEN	DRY	TOTAL N AS 100%
	%	%	%	%	%	%	%	%	%
Total N	0.4182	1.2810	100.00	0.2839	3.6750	100.00	0.5042	0.9761	100.00
Total organic N	0.3927	1.2029	93.90	0.2080	2.6922	73.27	0.4910	0.9506	97.38
Protein N	0.2173	0.6657	51.96	0.1539	1.9920	54.22	0.3768	0.7295	74.73
Soluble organic N	0.1754	0.5372	41.94	0.0541	0.7002	19.05	0.1142	0.2211	22.65
Cyanogenetic N	0.0136	0.0417	3.25	0.0048	0.0621	1.69	0.0446	0.0863	8.85
Amide N	0.0450	0.1378	10.76	0.0144	0.1864	5.07	0.0126	0.0244	2.50
Humin N	0.0133	0.0407	3.18	0.0012	0.0155	0.42	0.0070	0.0136	1.39
Total amino N	0.0773	0.2368	18.48	0.0276	0.3572	9.72	0.0326	0.0631	6.47
Basic-free amino N	0.0566	0.1734	13.53	0.0152	0.1967	5.35	0.0154	0.0298	3.05
Basic N	0.0468	0.1433	11.19	0.0168	0.2174	5.92	0.0320	0.0620	6.35
Ammonium N	0.0121	0.0371	2.89	0.0030	0.0388	1.06	0.0132	0.0256	2.62
Nitrate N	0.0134	0.0410	3.20	0.0729	0.9436	25.67	none	none	none
Other N	0.0001	0.0003	0.04	0.0017	0.0220	0.60	0.0026	0.0049	0.51

* Owing to the methods of analysis used with these two samples, the values presented for cyanogenetic, ammonium, and amide nitrogen may not be absolutely correct. Nevertheless any inaccuracies present are believed to be of minor importance.

no attempt has been made to ascertain the extent of varietal differences, the data show a wide range in the proportions of nitrogen found in the cyanogenetic, amide, amino, basic, and nitrate fractions.

Nitrates, for example, are not usually found in stems and leaves of peach trees grown in orchards in New Jersey, but they are sometimes present in small amounts in the tops of trees grown in sand cultures and supplied with nutrient solutions having high reactions and containing an abundance of this form of nitrogen. As shown in table VIII, the small non-woody roots of peach trees grown in sand cultures may contain considerably more nitrate than soluble organic nitrogen. When grown under orchard conditions, however, such roots seldom contain much nitrate nitrogen.

It is apparent that peach trees may exhibit wide differences in the distribution of their nitrogen. This is probably true for all plants. The significance of such differences in the peach cannot be considered here but will be dealt with in another publication.

Summary

1. No hydrocyanic acid was liberated when dormant or slowly growing peach stems were minced rapidly and boiled according to the method recommended by ROBINSON (**14**). With the same method of treatment the terminal portions of moderately or rapidly growing peach stems liberated very small to very appreciable quantities of hydrocyanic acid.

2. The optimum reaction for the enzymatic hydrolysis of the cyanogenetic glucosides in extracts of peach tissues is approximately pH 5.5 to 5.7. A reaction between pH 5.5 and 6.0 is satisfactory for most analytical purposes.

3. The cyanogenetic nitrogen present in peach extracts never was completely liberated during hydrolysis by boiling with various concentrations of sulphuric acid for different lengths of time.

4. During aspiration with 0.65 per cent. sodium hydroxide in the determination of ammonium nitrogen by the method of SESSIONS and SHIVE (**16**), cyanogenetic nitrogen may be more or less completely hydrolyzed to ammonium nitrogen. Because of this it was found necessary to remove cyanogenetic nitrogen as a first step in the analytical procedure in order to avoid inaccuracies in the determination of ammonium and amide nitrogen.

5. The addition of a soluble iron salt to a peach extract was found to prevent almost completely the hydrolysis of cyanogenetic nitrogen to ammonium nitrogen in an alkaline solution.

6. Considerably more ammonium nitrogen may be recovered from extracts of peach tissues by aspiration with 0.65 per cent. sodium hydroxide at room temperatures by the method of SESSIONS and SHIVE than by distillation *in vacuo* at 60° C. with a saturated solution of borax according to the method of WATCHORN and HOLMES.

7. When precautions were taken to destroy quickly the emulsin present in green peach tissues, no loss of nitrogen occurred during the drying of stem material. Under similar conditions of drying, root material lost a slight but not appreciable amount of nitrogen.

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CARBON DIOXIDE BALANCE AT HIGH LIGHT INTENSITIES

ELMER S. MILLER AND G. O. BURR

(WITH TWELVE FIGURES)

Introduction

In this first paper of a proposed series, it is shown that at a high light intensity all plants studied reached the same limiting CO_2 concentration and maintained this level for many hours of continuous illumination. Ten species of widely different types of leafy plants have been used, and at low temperatures these behaved alike. It appears, therefore, that there is an extremely narrow range of CO_2 concentration which so greatly limits the rate of photosynthesis that differences in rates of respiration and other internal factors have little effect on this point of balance between respired and assimilated CO_2.

Over a wide range this CO_2 balance is independent of temperature. At very high temperatures (36° C.), however, some species no longer maintain the usual CO_2 balance, but reach a much higher value (figs. 6, 8). This is especially emphasized here because of its relation to the abnormal photosynthetic behavior of plants reported recently by KOSTYTSCHEW and coworkers (**8, 9**).

Apparatus

Although much work has been done with land plants or single leaves, many workers in recent years have turned to the submerged plant because of the ease with which its conditions can be controlled. It is true that in photosynthetic work with high light intensities, leaves or whole plants are subjected to abnormal conditions when inclosed in a glass vessel through which a very slow stream of air is drawn. This makes for abnormally high internal temperatures and humidities because of restricted air cooling and evaporation.

The important feature of the apparatus used for this work is the closed system which permits a continuous and vigorous circulation of air about the plant (fig. 1). This makes possible an easy control of the humidity, temperature, and evaporating power of the air. The leaves may be raised to a high temperature while the roots are kept cool, thus maintaining the plant in an environment which is not unusual. The simple apparatus pictured in figure 1 makes possible a wide range of temperature and humidity. The air temperature can easily be held constant to within $\pm 1°$ C. over a period of 12 hours or more without attention. In this work the temperature has been varied from 4° to 37° C. and there is no reason why a still wider

range cannot be just as easily attained. Atmometer readings and transpiration records indicate that the evaporating power of the air has been kept fairly high. The only means of lowering humidity in these experiments has been the condensation of water in the cooled parts of the apparatus. A further reduction of humidity can be accomplished by the insertion of a drying agent in the circulating air stream. Work along this line is being undertaken.

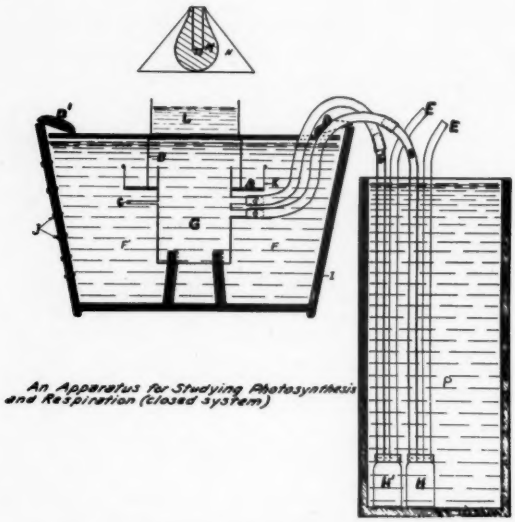


FIG. 1. Photosynthesis chamber and connections.

The plant chamber (fig. 1 *G*) is constructed of tinned copper. This permits ready temperature control in conjunction with the copper tubes immersed in the ice bath (*P*). The pump circulates 10–12 liters of air per minute.

Method of analysis

The carbon and oxygen were determined with the HALDANE-CARPENTER (**4**) apparatus. All analyses were made in a sub-basement where the experiments were conducted, a place that is only slightly affected by outdoor temperature fluctuations. After preliminary and outside air analyses had been made to check the apparatus, the sample was drawn directly from the chamber through a capillary tube. After washing back and forth five times the sample was taken. Employing this technique, two duplicate determinations can be made to check within the experimental error involved in the manipulation of the machine. After each set of analyses, the capil-

lary tube was opened to the room air for approximately one second to permit an equilibrium with the gases in the plant chamber. Each analysis requires 40 cc. of air; therefore 40 cc. of outside air containing approximately 0.013 cc. of CO_2 were introduced after each analysis. This is negligible.

In this apparatus with a total volume of 8.33 liters for plant chamber, coils, and pump, a gain or loss of 1 cc. of either carbon dioxide or oxygen causes a 0.012 per cent. change in the concentration of the respective gases. For carbon dioxide, the HALDANE-CARPENTER apparatus has an accuracy of $\pm$ 0.001 per cent. or $\pm$ 0.085 cc. of this gas taken up or given off by the plant. For oxygen it has about one-third this accuracy.

In some tests on rates of respiration (experiments 107, 111, and 112, table II), only 13 to 20 mg. of carbon dioxide were evolved; therefore the maximum carbon dioxide error is 2.3 per cent. in studies of these respiratory rates. The percentage error can be greatly reduced by continuing the experiment over a longer period.

Material

For this investigation, potted greenhouse plants of a size that would conveniently fit into the plant chamber were used. The types of plants used were one hydrophyte, seven mesophytes, and two xerophytes. The purpose of using such a variety was to ascertain whether plants with leaves of different thicknesses and characteristic of different habitats responded to conditions in a similar manner. The plants were brought from the greenhouse to the laboratory two hours preceding the experiment. During this period the plants were kept in almost total darkness and at a temperature of approximately 20° C. At the beginning of the experiment, the plants were watered with 30 cc. of water. At the end of each experiment the widths and the lengths of the leaves were measured. From these data from a special set of graphs designed for each species the respective leaf areas were calculated.

Before the use of potted plants was decided upon, a number of experiments were conducted on cut *Pelargonium* stems with adventitious roots 4 to 9 cm. long. These plants were kept in a Shive's nutrient solution (one-third usual concentration). Particular pains were taken to note the temperature of this culture solution at the end of each experiment, and in no case did it exceed 18° C. when the air about the leaves was 35°–37°. Hence even at the higher temperature one was sure that the roots did not suffer from overheating.

When these experiments at 4°–6° and 35°–37° C. were completed, potted *Pelargonium* plants were used and it was found that the curves for the latter were similar to those obtained on the cut stems. It was therefore

concluded that the soil bacteria and the plant roots themselves introduced no appreciable error. It was felt that for metabolism studies extending over many hours, potted plants rather than cut stems would give results more characteristic of the species in its native habitat.

Procedure for CO_2 steady states

The level and temperature of the water were adjusted according to previous experience to give the desired temperature about the leaves. The air in the room was renewed with a large ventilator so that the CO_2 content was less than 0.035 per cent., and the apparatus was filled with this air by running the pump with the chamber lid off. The plant to be studied was then put inside, the chamber closed, the pump started, and the air brought to temperature while the plant was in darkness. The first air sample was drawn for analysis and the light turned on. Since the apparatus was being used as a closed system, rise or fall in percentage of CO_2 and O_2 was a measure of rate of gas exchange, volume being known (0.012 per cent. change = 1 cc. gas).

In experiments 1–10 inclusive the light from a 1000-watt mazda lamp was filtered through 5 cm. of water. The intensity inside the plant chamber was 2050 foot candles (measured with a MacBeth illuminometer). All plants chosen had their leaves spread in a horizontal plane so that they did not shade each other appreciably, and they were approximately the same distance from the light. Each curve shown in figures 2–11 came from a different individual plant. Experiments 1a, 1b, 1g, and 1h were conducted with the cut *Pelargonium*; the others with potted plants.

Duplicate analyses were usually made for each point on the curves.

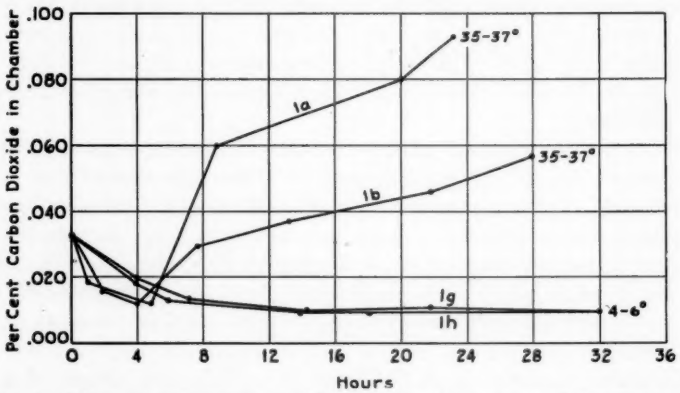


FIG. 2. Effect of temperature on CO_2 exchange of cut stems of *Pelargonium hortorum* Bailey. Light intensity = 2050 f.c.

Results

The leaf area, transpiration, and atmometer values for the 34 plants used in experiments 1–10 are given in table I.

Figures 2–12 show the course of CO_2 exchange following exposure of the plants to 2050 f.c. light intensity. Every plant at some temperature quickly reduces the CO_2 concentration in the chamber to 0.01 volume per cent., a level which is maintained over long periods of time (24 hours or more). No

TABLE I

AMOUNTS OF EVAPORATION AND TRANSPIRATION OF THE PLANTS USED IN EXPERIMENTS 1A TO 10B INCLUSIVE AT DIFFERENT TEMPERATURES

EXPERIMENT NO.	TEMPERATURE	TRANSPIRATION	DURATION	LEAF AREA	EVAPORATION FROM ATMOMETER	TRANSPIRATION/100 CM.² LEAF AREA/24 HOURS
	° *C.*	*gm.*	*hrs.*	*cm.²*	*gm.*	*gm.*
	Pelargonium hortorum					
1a	35–37	17.0	23.0	388	.....	4.57
1b	35–37	21.5	28.0	260	.....	7.09
1c	35–37	47.5	26.0	356	64.80	12.32
1d	35–37	40.0	26.0	237	63.52	15.58
1e	4–6	7.0	32.0	202	14.16	2.60
1f	4–6	7.8	32.0	253	13.60	2.31
1g	4–6	12.7	32.0	406	.....	2.35
1h	4–6	14.5	32.0	348	.....	4.17
	Tolmiea menziesii					
2c	35–37	125.5	38.5	471	46.88	16.61
2d	35–37	128.7	33.0	317	43.52	29.34
2a	22–23	73.8	36.0	378	29.28	13.02
2b	22–23	51.7	24.0	349	26.96	14.81
	Coleus blumei var. *verschaffeltii*					
3a	35–37	33.0	21.25	347	14.40	10.74
3b	35–37	59.5	23.0	321	16.20	19.34
	Bryophyllum pinnatum					
4a	35–37	33.6	15.8	146	17.76	34.96
4b	35–37	56.0	20.8	272	20.16	23.75
4c	6–8	7.5	24.0	170	12.48	4.41
4d	6–8	8.7	25.0	222	12.96	3.76
	Eichhornia crassipes					
5a	35–37	330.0	65.3	182	.....	66.63
5b	35–37	155.0	38.0	161	.....	58.84

TABLE I (*Continued*)

Experiment no.	Temperature	Transpiration	Duration	Leaf area	Evaporation from atmometer	Transpiration/100 cm.² leaf area/24 hours
	° *C.*	*gm.*	*hrs.*	*cm.²*	*gm.*	*gm.*
	Crassulaceae sp.*					
6a	35–37	92.3	69.0	234	.....	13.72
6b	35–37	62.5	47.0	295	.....	10.82
6c	4–6	8.8	24.0	234	.....	3.76
6d	4–6	9.0	24.0	295	.....	3.05
	Primula auricula					
7a	35–37	22.3	24.0	567	29.20	3.93
7b	35–37	49.3	23.5	530	28.96	9.50
	Saxifraga sarmentosa					
8a	35–37	74.3	21.5	574	29.20	13.22
8b	35–37	29.4	22.0	386	32.0	7.54
	Zebrina pendula					
9a	35–37	29.2	23.0	297	.....	10.26
9b	35–37	40.0	23.0	548	.....	7.62
9c	16–18	22.0	22.0	297	.....	7.53
9d	16–18	24.2	23.5	548	.....	4.51
	Begonia tuberhybrida					
10a	35–37	54.5	22.5	199	.....	29.21
10b	35–37	48.5	22.5	613	.....	8.44

* It is regretted that, since no fruits of this plant were available, the taxonomists at Minnesota were unable to identify it further for us.

CO_2 is being introduced into the chamber from the outside; it is a closed system in which a horizontal curve indicates that CO_2 respired equals CO_2 assimilated. The slope of a rising curve gives the rate of respiration in excess of photosynthesis and of a falling curve the excess photosynthesis.

The advantage of studying CO_2 balance rather than rates of CO_2 assimilation is forcibly shown by an examination of the curves in figures 2–12. The assimilation rates, as shown by the downward slopes of the curves after the start of the experiment, vary considerably among individuals of the same species and among species. When the total CO_2 assimilated per hour is divided by the leaf area (table I), values ranging from 0.42 to 1.32 cc. CO_2/100 cm.²/hr. are found. Yet these plants all come to the same point of balance.

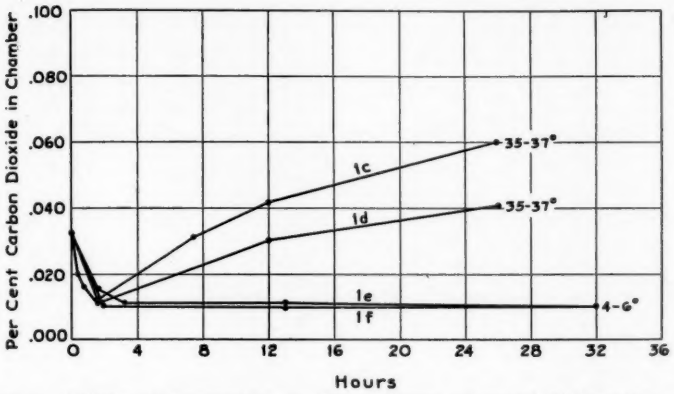


FIG. 3. Effects of temperature on CO_2 exchange of potted *Pelargonium* plants. Light intensity = 2050 f.c.

The reason for this irregularity in rate of assimilation seems clear. Rate is dependent partly upon CO_2 diffusion from air to chloroplast which in turn is regulated by stomal numbers and degree of opening, arrangement of cells and air spaces within the leaf, viscosity of solution within the cell, arrangement of chloroplasts, and other internal factors. MASKELL (**11**) has given a detailed discussion of internal resistances.

The following points are of special interest:

1. The curves break fairly sharply as 0.010 per cent. CO_2 is approached.
2. The limiting percentage of CO_2 is independent of temperature provided the temperature is not high enough to cause abnormal metabolism (fig. 6).

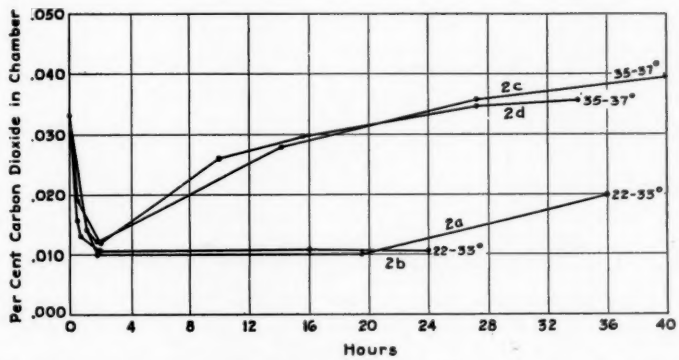


FIG. 4. Effect of temperature on CO_2 exchange of *Tolmiea menziesii* T. & G. Light intensity = 2050 f.c.

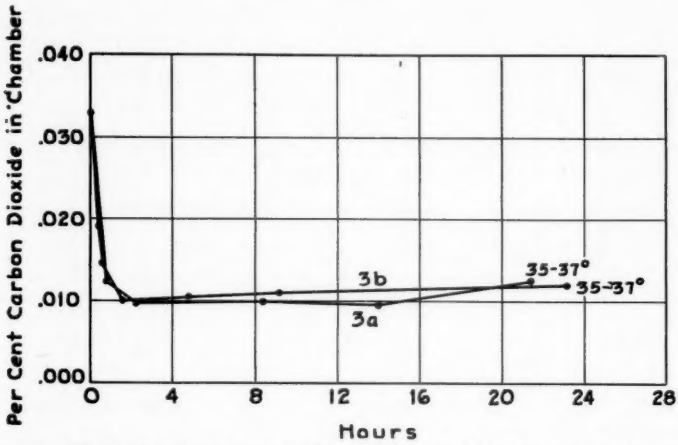


FIG. 5. Effect of temperature on CO_2 exchange of *Coleus blumei* Benth. var. *verschaffeltii* Lem. Light intensity = 2050 f.c.

3. The limiting percentage of CO_2 is independent of leaf properties (thickness, chlorophyll content, sap constants).

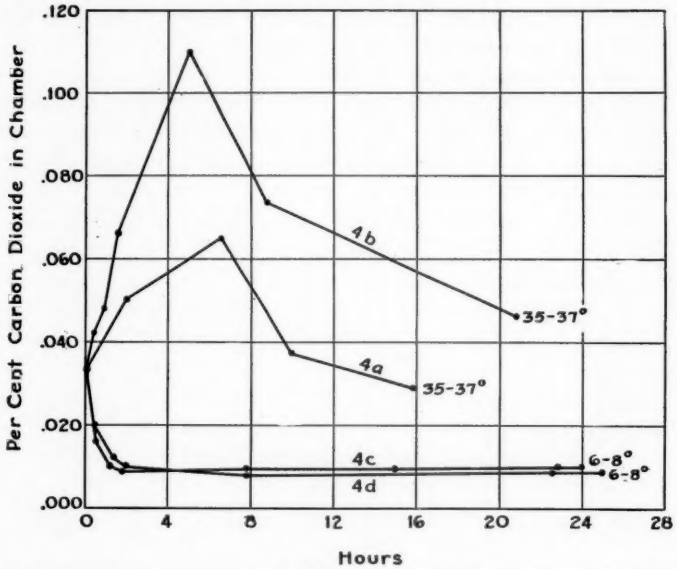


FIG. 6. Effect of temperature on CO_2 exchange of *Bryophyllum pinnatum* Kurz. Light intensity = 2050 f.c.

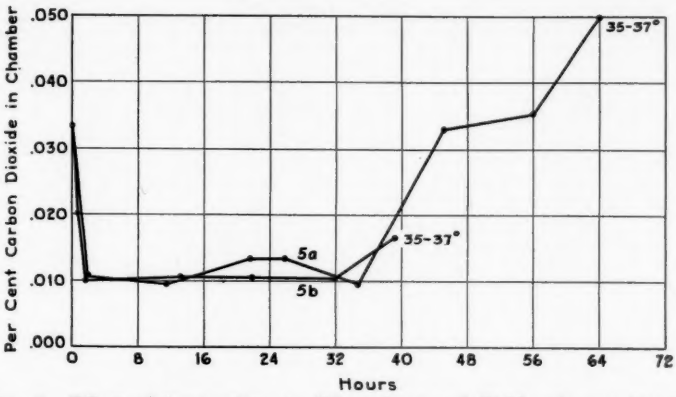


FIG. 7. Effect of temperature on CO_2 exchange of *Eichhornia crassipes* Solms. Light intensity = 2050 f.c.

4. Balance in CO_2 exchange does not necessitate a balance in O_2 exchange.

5. Plants may be segregated into definite groups according to their behavior at high temperatures.

1. The sharp breaks of many of the rate curves as the CO_2 concentration approaches 0.01 per cent. are excellent examples of close approximation to BLACKMAN'S (**2**) diagrammatic picture of limiting factors. The available CO_2 in the closed system is quickly consumed at a high rate of apparent assimilation (assimilation in excess of respiration) until the region of CO_2 limitation is reached, at which apparent assimilation becomes zero (respira-

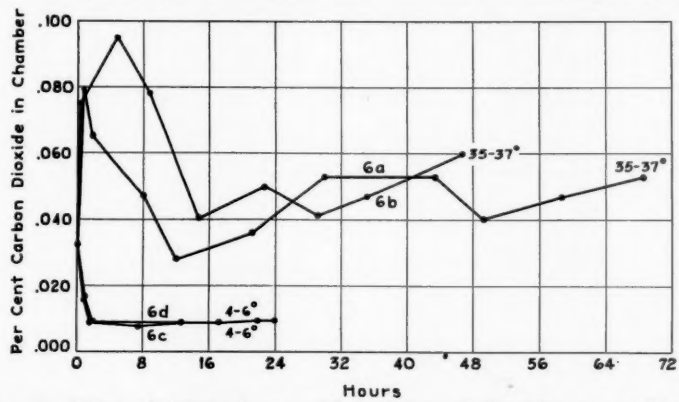


FIG. 8. Effect of temperature on CO_2 exchange of Crassulaceae sp. Light intensity = 2050 f.c.

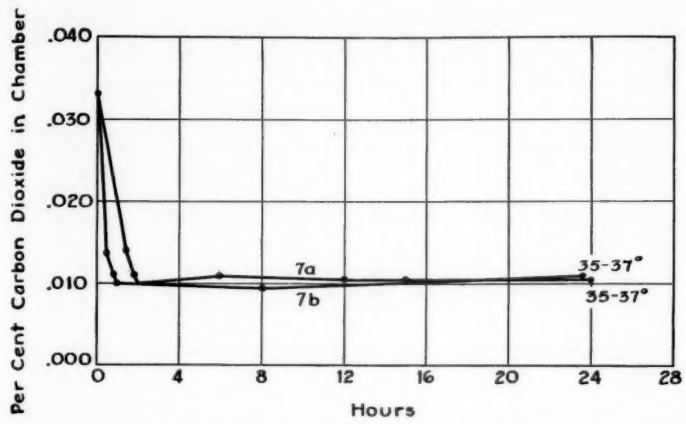


FIG. 9. Effect of temperature on CO_2 exchange of *Primula auricula* L. Light intensity = 2050 f.c.

tion = photosynthesis). These findings are in almost perfect agreement with two of VAN DEN HONERT's (6) curves, which show that at 0.010 volume per cent. CO_2 is the chief limiting factor while at 0.015 volume per cent. it no longer exerts any great influence on the rate of assimilation.

The transition zone is very narrow. It is this narrowness which may partially account for the apparently uniform behavior of all plants studied. Much of the change in slope of the rate curve may take place in a range of 0.005 per cent. CO_2. And if, after reaching 0.010 per cent. CO_2, we con-

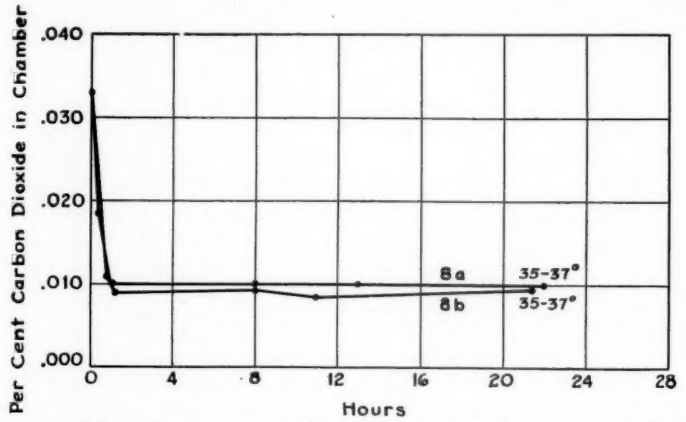


FIG. 10. Effect of temperature on CO_2 exchange of *Saxifraga sarmentosa* L. Light intensity = 2050 f.c.

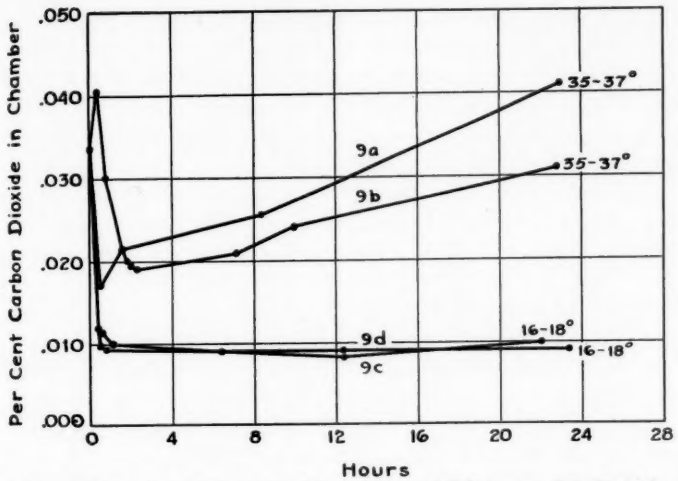


FIG. 11. Effect of temperature on CO_2 exchange of *Zebrina pendula* Schnizl. Light intensity = 2050 f.c.

sider the rate of assimilation directly proportional to CO_2 concentration, a change of 0.002 per cent. will change the rate 20 per cent. Since the minimum error of our analyses is $\pm$ 0.001 per cent., it is evident that very appreciable differences might exist between the plants without our detecting them.

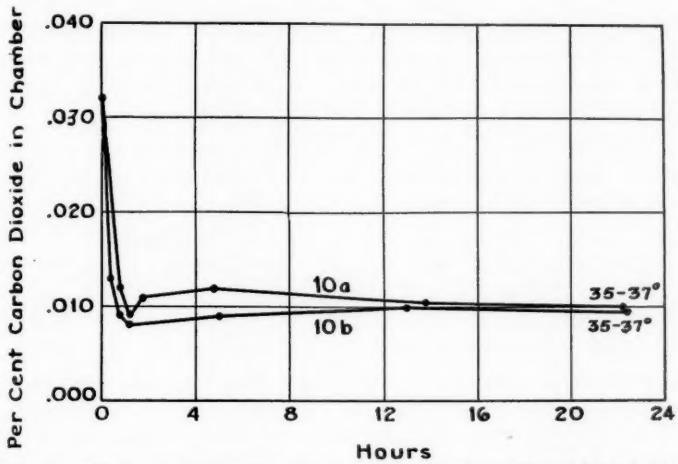


FIG. 12. Effect of temperature on CO_2 exchange of *Begonia tuberhybrida* Voss. Light intensity = 2050 f.c.

It is well known, however, that different plants vary widely in their absolute respiration rates. The rates for some of our plants are given in table II. One *Primula* plant respired at more than twice the rate of one *Pelargonium*. Yet both reach the same CO_2 balance. This suggests that there is a close relationship between ability for respiration and for assimilation, an idea which is not new.

SPOEHR and MCGEE (**18**) state: "It now seems highly probable that a solution of the problem of the internal factor in photosynthesis can be found in the intimate interrelation between photosynthesis and respiration" (p. 76).

WARBURG (**22**) concluded that the intermediates of respiration might be reutilized in photosynthesis more readily than carbon dioxide.

VAN DER PAAUW (**13**) concluded that there is a close parallelism between photosynthesis and respiration. Mild stimulation or retardation of both processes can be accomplished by KCN. This does not agree with earlier work of WARBURG (**22**). ARNOLD (**1**), however, found that "an unidentified unit in the mechanism of photosynthesis of *Chlorella pyrenoidosa* is rendered inactive by absorption of one quantum of ultra-violet light (2537 Å wave length)" without an appreciable effect on the normal respiration.

0.01 per cent. CO_2 does not represent the limit to which a cell may reduce its CO_2 tension by photosynthesis. The curves here given are for whole potted plants with leaves of varying thickness. Cells on the lower side of thick leaves are in very dim light and their respired CO_2 must be utilized by the lighted cells. There is also the CO_2 from stem, roots, and soil to be taken into account. Therefore 0.01 per cent. may be considered the maximum value to which a whole plant can raise the CO_2 in a closed system when its leaves are in light of 2000 f.c. intensity.

2. It has been found that the CO_2 balance is not altered by considerable changes in temperature. A plant may be kept at 5° or at 36° C. and in both cases reduce the CO_2 to 0.01 per cent. Since at this point respiration equals photosynthesis, the rates of both must increase equally. It is shown in table II that respiration rate in the dark increases about fourteen or fifteen times for both *Primula* and *Begonia*. These species both maintain the 0.01 per cent. level of CO_2 at 36° as well as at 5° (figs. 9, 12). Assuming the same temperature coefficient for respiration in the light, we must conclude that the rate of photosynthesis likewise increased about fifteen times over this same range in temperature. As these steady states were maintained for more than 20 hours, it is evident that the two rates were always identical, for the slightest difference would soon raise or lower the CO_2 concentration a measurable amount.

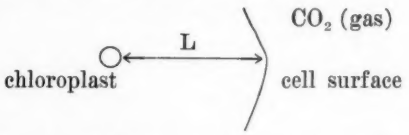
TABLE II

RESPIRATORY QUOTIENTS AND RATES OF RESPIRATION

EXPERIMENT NO.	PLANT	TEMPERATURE	CO_2 RESPIRED	LEAF AREA	RESPIRATORY QUOTIENT	CO_2 RESPIRED/M^2 LEAF AREA/HOUR	RATE, 35°–37° / RATE, 4°– 6°
		°C.	%	$cm.^2$		mg.	
101	*Pelargonium*	35–37	0.945	200	0.85	735.35	57
107		4–6	0.036	300		12.79	.....
102		35–37	1.361	297	0.80	515.79	
108		4–6	.....	297		.....	
103	*Primula*	35–37	0.362	397	0.78	265.92	14
109		4–6	0.082	397		19.54	
104		35–37	0.969	376	0.80	421.54	15
110		4–6	0.073	376		28.14	
105	*Begonia*	35–37	0.806	560	0.79	243.91	15
111		4–6	0.100	560		16.12	
106		35–37	0.551	298	0.78	263.18	14
112		4–6	0.062	298		18.47	

This is further evidence that light is in excess, and that in assimilation, the rate determining step is not the primary process involving light absorption but an intermediate dark reaction limited by CO_2 concentration. Probably the temperature coefficient of this reaction is very close to that of respiration and it may be identical with it. This view is in accord with the recent findings of VAN DER PAAUW (**13**, p. 564), by whom the Q_{10}'s for photosynthesis and respiration were shown to be almost identical over the range 10°–30° C. The experimental method employed by investigators of CO_2 balance is especially good because it eliminates much of the diffusion resistance and permits the comparison of photosynthesis and respiration in the same leaf at the same time.

A real difficulty arises when a mechanical picture of the utilization of respired CO_2 is considered. The following diagram illustrates the relation between CO_2 and the chloroplast within the cell:



CO_2 (gas) is in equilibrium with CO_2 (dissolved) at the surface of the cell. Let C_1 be the concentration of CO_2 (dissolved) at the cell surface and C_2 the concentration at the chloroplast surface. Then the rate of diffusion of respired CO_2 from surface protoplasm to chloroplast surface is proportional to $\frac{C_1 - C_2}{L}$, where L is the distance. When the temperature was raised 31° the respiration in the dark increased about fifteen times. In order that the respired CO_2 at the cytoplasm surface reach the chloroplast fifteen times as fast, the gradient $\frac{C_1 - C_2}{L}$ must increase fifteen times[1] The surface concentration, C_1, does not increase since there is no rise in the CO_2 (gas). If the distance, L, remain constant then the only way to increase the gradient would be by a drop in C_2. An appreciable drop in CO_2 does not seem likely since assimilation shows the expected increase in rate with temperature rise.

The simplest explanation of the utilization of the increased products of respiration without an increase in CO_2 tension seems to be that an intermediate product of respiration is reutilized before any CO_2 is liberated. This agrees with the suggestion, previously stated, that there is a very

[1] VAN DEN HONERT (**6**) has pointed out that fall in viscosity is about equal to the fall in solubility of CO_2 with rise in temperature. Therefore, when the diffusion of CO_2 is the chief limiting factor in assimilation a Q_{10} of 1 is to be expected. His experimental data support this view. Hence the diffusion factor is eliminated from our consideration.

close relationship between photosynthesis and respiration, and is in line with the suggestion of WARBURG (**22**) that possibly an intermediate product of respiration is utilized in photosynthesis when no CO_2 is being exchanged by the plant.

3. The balance between respiration and photosynthesis is reached at a definite CO_2 tension. No measurements of pH were made on the leaves studied, but it is highly improbable that all ten species had the same hydrogen ion concentration at the time of the experiment. Without assuming any definite pK'_1 for the cell saps, on rearranging the Henderson-Hasselback equation to

$$\log \frac{\text{combined } CO_2}{\text{dissolved } CO_2} = pH - pK'_1$$

it becomes clear that there is a fixed ratio of combined to dissolved CO_2 when $pH - pK'_1$ is a constant. It is not likely that all plants used in this investigation assumed such a fixed relation between pH and pK'_1. When $pH - pK'_1 = -0.3$ dissolved CO_2 is twice combined CO_2; when $pH - pK'_1 = +0.3$ combined CO_2 is twice dissolved CO_2.

If these assumptions are correct, at constant CO_2 tension the different plants differed widely in combined CO_2, and it may be concluded that the steady state observed is a function of dissolved CO_2 and that the rate of photosynthesis is limited by the concentration of H_2CO_3 and not by bicarbonates.

This view is in accord with the theory of WILLSTÄTTER and STOLL (**23**, p. 244) for the combining of H_2CO_3 with chlorophyll. It is probable that $NaHCO_3$ could not enter into this reaction. JAMES (**7**), VAN DEN HONERT (**6**), and others have assumed that only dissolved CO_2 is available for assimilation.

4. In experiments 101–106 (table II) the total gas exchange is great so that the respiratory quotients are of high accuracy. Their values range from 0.78 to 0.85. These are in agreement with the values found by MAQUENNE and DEMOUSSY (**10**) for young leaves after a few hours in darkness. Our plants were in the chamber several hours at the high temperatures, hence the low respiratory quotients are to be expected.

If we accept the value of 1.00 for photosynthetic quotient, it is evident that when CO_2 is in balance O_2 will be disappearing, *i.e.*, the plants are not in energy equilibrium. These respiration experiments were continued through a period of CO_2 equilibrium. While the change in CO_2 concentration was zero, the O_2 changed appreciably (table III).

This brings up an interesting question in regard to compensation point. PLAETZER (**14**) defined compensation point as that light intensity at which gas exchange in a leaf is zero. Neither gas was specified. PLAETZER and

TABLE III

CHANGE IN CONCENTRATION OF OXYGEN AND CARBON DIOXIDE AFTER CO_2 EQUILIBRIUM HAD BEEN MAINTAINED SEVERAL HOURS

EXPERIMENT NO.	PLANT	TEMPERATURE	DURATION	CHANGE IN CO_2 CONCENTRATION	CHANGE IN O_2 CONCENTRATION IN 5 HOURS
		° *C.*	*hr.*	%	%
101	*Pelargonium*	35–37	5.071	+0.002	−0.105
102	*Pelargonium*	"	6.071	−0.001	−0.077
103	*Primula*	"	6.000	+0.002	−0.079
104	*Primula*	"	5.500	± 0.000	−0.087
105	*Begonia*	"	4.500	± 0.000	−0.077
106	*Begonia*	"	4.500	+0.001	−0.084
107	*Pelargonium*	4–6	5.750	± 0.000	−0.012
108	*Pelargonium*	"	4.750	+0.001	−0.053
109	*Primula*	"	4.500	± 0.000	.....
110	*Primula*	"	8.000	−0.001	−0.053
111	*Begonia*	"	5.000	−0.001	−0.053
112	*Begonia*	"	4.500	± 0.000	−0.038

HARDER (**5**) have used O_2 exchange as the measure, while BOYSEN-JENSEN (**3**), MÜLLER (**12**), and others follow CO_2 exchange. Since we have reason to believe that O_2 consumption and CO_2 evolution in respiration are distinct reactions with different characteristics (**19–21**), it would be well to specify which gas is to be in balance at the compensation point.

5. An examination of figures 2–11 shows that plants exhibit a great variability in their behavior at 35°–37° C. They may be classified into three general groups: (1) Those which are stable at 35°–37° and reach the same point of CO_2 equilibrium as shown at lower temperatures (*Coleus, Eichhornia, Primula, Saxifraga, Begonia*); (2) those which temporarily follow the low temperature curve and then begin active CO_2 evolution (*Pelargonium, Tolmiea, Zebrina*); (3) those which evolve large quantities of CO_2 as soon as they are raised to 35°–37° and do not in the course of the run reduce the CO_2 concentration below that of normal air (*Bryophyllum* and Crassulaceae sp.).

These curves show the danger in working at such high temperatures when studying CO_2 assimilation, and probably explain some of the recent results of KOSTYTSCHEW and co-workers (**8, 9**), who have carried out extensive investigations of the daily course of photosynthesis of plants of central Asia. They inclosed the attached leaf in a glass chamber in the field and with an aspirator drew a small volume (about 11 liters) of air across the leaf. Most of their runs did not exceed 30 minutes. The temperature inside the chamber was not recorded, but the outside temperature was often

TABLE IV

CHANGES IN CONCENTRATION OF O_2 AND CO_2 IN PLANT CHAMBER DURING PERIOD OF MAXIMUM GASEOUS EVOLUTION

EXPERIMENT NO.	PLANT	TIME AFTER START OF EXPERIMENT							
		3 HOURS		4 HOURS		5 HOURS		6 HOURS	
		CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
		%	%	%	%	%	%	%	%
4a	*Bryophyllum*	+0.019	+0.082	+0.013	+0.071	.....	.....	.....	.....
4b	*Bryophyllum*	+0.057	+0.017	+0.055	+0.020	.....	.....	.....	.....
6a	Crassulaceae sp.	+0.032	+0.070	+0.025	+0.068	.....	.....	.....	.....
6b	Crassulaceae sp.	.....	.....	.....	.....	+0.056	+0.056	+0.054	+0.040

as high as 37°. In bright light there was undoubtedly a considerable rise of leaf temperature within the chamber. KOSTYTSCHEW and his colleagues observed sharp fluctuation in photosynthesis, especially by the xerophytes in the Bogar plots. There were short flashes of photosynthesis alternated with short but energetic evolution of carbon dioxide in the light.

It is evident from figures 2–11 that at high temperatures almost any kind of value for CO_2 assimilation might be obtained with the different species, and that a 30-minute run made immediately after raising the temperature does not give a true picture of CO_2 exchange. For example, during the first two hours *Pelargonium* shows a high rate of photosynthesis and Crassulaceae sp. a high rate of CO_2 evolution, but at the eighth hour *Pelargonium* is evolving CO_2 and Crassulaceae sp. is carrying on rapid photosynthesis. At lower temperatures, however, all plants show uniform photosynthesis curves.

There are at least two kinds of CO_2 evolution shown in figures 2–11. That which is evolved immediately (figs. 6, 8) must be present in the leaf as dissolved and combined CO_2 (including carboxylic acids) which is released by the rise in temperature and high light intensity. That CO_2 which begins coming off only after several hours at the higher temperature (figs. 2, 3, 4, 11) probably represents CO_2 of metabolism after the high temperature has caused metabolic readjustments within the leaf.

In order to show that the carbon dioxide which came from *Bryophyllum* and Crassulaceae sp. was not produced by respiration suddenly speeded up, those runs were repeated and both oxygen and carbon dioxide were determined. The results are given in table IV. Since both gases showed marked increment, the increase in CO_2 cannot be attributed to normal aerobic respiration.

One hundred cc. of distilled water dissolves from air at 760 mm. and 20° C. 0.026 cc. CO_2 and 0.649 cc. O_2; at 35° C. 0.018 cc. CO_2 and 0.501 cc. O_2. Assuming the same change in solubility in leaf sap, a rise of 15° C. would liberate only 0.148 cc. O_2 from 100 cc. of leaf tissue, so that the

TABLE V

EXPERIMENT NO.	PLANT	CO_2 EVOLVED PER GM. DRY LEAF
4a	*Bryophyllum*	4.64
4b	*Bryophyllum*	5.06
6a	Crassulaceae sp.	3.97
6b	Crassulaceae sp.	2.33

solubility factor is negligible. The oxygen most likely comes from photosynthesis and table IV indicates that O_2 increases at the expense of CO_2 (experiments 4a and 6a). Therefore the total CO_2 evolved would really be equal to $CO_2 + O_2$.

Table V gives the calculated CO_2 liberated by the leaf material at the time of greatest $CO_2 + O_2$ increase measured.

These are much larger values than found by SPOEHR and MCGEE (**18**) for the total CO_2 combining power of dried leaves, excepting sunflower (13.9) and nettle (6.2). The release of all combined (bicarbonate) CO_2 requires a large increase in acidity (pH 4.5) which probably does not take place. It seems likely, therefore, that much of the CO_2 liberated at high temperatures arises through a fermentation type of respiration, including decarboxylation.

It should be pointed out that when plants are in CO_2 equilibrium with the air, there is little possibility of their holding very considerable quantities of either free or combined CO_2 (as bicarbonates). In the Henderson-Hasselback equation

$$pH = pK'_1 + \log \frac{\text{combined } CO_2}{\text{dissolved } CO_2}$$

no definite value can be set for pK'_1. Its value for sea water is given by SAUNDERS (**16**) as 6.06 and the accepted value for blood is 6.1. If a value of 6.0 is assigned to the average plant, then at pH 6.0 the volume of combined CO_2 would be equal to dissolved CO_2. Recorded values indicate that most plant saps are rarely more alkaline than pH 6. Therefore with a maximum possible CO_2 solubility at 20° C. of 0.026 cc. per 100 cc. of leaf sap, the total CO_2 in the leaf could rarely be more than 0.05 cc. per 100 cc. of plant sap. This limiting value would not hold if gaseous CO_2 were trapped within the leaf at a concentration above that found in air.

RICHARDS (**15**) observed very high rates of CO_2 evolution when joints of cactus (*Opuntia versicolor*) were exposed to light. In diffuse light (intensity not stated) the rate of CO_2 evolution was about the same as in the dark, but very little O_2 was absorbed so that the respiratory quotient rose to values as high as 7.00. In direct sunlight, CO_2 was evolved at a somewhat lower rate and O_2 came off at the same time. RICHARDS likewise observed that when there was high CO_2 evolution there was low O_2 evolution. The results of our work at high temperatures with *Bryophyllum* and Crassulaceae sp. described in this work are in close agreement with the work of RICHARDS. He attributes the high CO_2 production in light to the photochemical decomposition of organic acids, and shows a large decrease in total acidity to support this view. The runs in which both O_2 and CO_2 egressed were made at high temperatures (30°–39° C.), and RICHARDS

realized that this was probably an important factor. The normal photosynthetic behavior of *Bryophyllum* and Crassulaceae sp. at 4°–6° C. substantiates his suggestion that *Opuntia* stores energy only in cooler seasons, while it grows at the expense of this stored food in hot weather. SPOEHR (**17**) has further discussed the photolysis of organic acids in the cactus.

In view of the results here reported, it must be concluded that temperature is fully as important as light in determining the rate of evolution of CO_2 from plants exposed to light. In fact the photosynthesis curves of *Bryophyllum* and Crassulaceae sp. at 4°–6° C. are so similar to the curves of other leaves that it would appear that no decarboxylation takes place in 2000 f.c. light intensity at the low temperature. That temperature is the chief factor causing the evolution of CO_2 by *Pelargonium* is shown by the enormous increase (fifty-seven times) in dark respiration of this species with a 31° rise in temperature (table II, experiments 101 and 107).

KOSTYTSCHEW, *et al.* observed the greatest CO_2 evolution by xerophytes of central Asia. The work of RICHARDS with *Opuntia* and the work here reported, combined with that of KOSTYTSCHEW, would point to a specialized metabolism not merely of succulents but of xerophytic plants generally. The recent work of TANG (**19–21**) again strongly emphasizes the view that CO_2 evolution and O_2 consumption must be considered as separate processes with different mechanisms and specific characteristics.

Summary

Under the conditions of these experiments the following conclusions may be drawn:

1. Many types of potted plants in a closed system quickly reduce the CO_2 to 0.01 volume per cent. and maintain this concentration in light of about 2000 f.c. intensity.

2. The sharp break in the rate curve indicates a close approximation to BLACKMAN'S ideal graph for limiting factors.

3. CO_2 balance is independent of temperature, suggesting that a dark reaction of photosynthesis has a temperature coefficient equal to the temperature coefficient of respiration.

4. CO_2 balance depends on the concentration of dissolved CO_2 and not on total CO_2 (H_2CO_3 plus bicarbonates).

5. Plants in CO_2 balance are not in an energy balance because the respiratory quotient under many conditions is not unity.

6. Evolution of CO_2 from succulents and xerophytes in bright light is caused by high temperature rather than by light.

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OSMOTIC QUANTITIES OF PLANT CELLS IN GIVEN PHASES[1]

ALFRED URSPRUNG

(WITH TWO FIGURES)

I. Osmotic quantities to be distinguished

Some authors use seven or eight terms, others content themselves with two or even a single one. The points of view are much divided. In order to reach an objective judgment, let us consider a cell from the pith of *Impatiens noli-tangere,* a cell which has been accurately measured by URSPRUNG and BLUM (**48**), MOLZ (**28**), URSPRUNG and BECK (**44**), and URSPRUNG (**46**). Cf. also BECK (**1**). Let V represent the volume of the given cell, and the indices n, g, and s, represent the normal phase, incipient plasmolytic phase (grenzplasmolytischen), and saturation phase respectively. We distinguish (fig. 1, schematic sketch of cell) the normal volume ($V_n = 14{,}122$ units) of the unchanged cell, the volume at incipient plasmolysis ($V_g = 13{,}209$ units), and the volume at complete saturation ($V_s = 14{,}779$ units). V_n had at the time of observation the value just given, which, however, changed as the water balance within the cell changed.

If we desire to measure the osmotic potential of the cell sap of the individual cell, we must begin with the phase of incipient plasmolysis. By means of the plasmolytic method we find first that the incipient plasmolysis value (*i.e.,* the osmotic value at incipient plasmolysis) is $O_g = 0.38$ mol cane sugar. This concentration of the cane sugar solution will cause the protoplasm of our cell to recede from the cell wall ever so little. In the absence of complicating factors, so that the volume changes of the cell have no other effects than corresponding changes in the concentration of the cell sap, one can calculate the osmotic value of the normal sap, O_n, from $O_g = 0.38$ by the equation: $O_n = O_g \frac{V_g}{V_n} = 0.355$ mol cane sugar. If we place the cell sap or an isosmotic cane sugar solution in an osmometer with a semipermeable membrane, the cell sap at incipient plasmolysis would develop an osmotic pressure (physicist's terminology) or a suction force (suction tension, suction[2]) (our terminology[3]) of 10.5 atm. In the condition of equilibrium the protoplasm must have the same suction force. Hence we may write: *The suction force (suction tension, suction) of the contents of the cell in the phase of incipient plasmolysis* is $S_{1g} = 10.5$ atm. Similarly the suction force (suction tension, suction) of the contents of

[1] Translated by WILLIAM A. BECK.

[2] Original: Saugkraft (Saugspannung, Saugung).

[3] The pros and cons of the matter of terminology will be discussed later.

the cell in the normal phase, S_{1_n}, or since for the sake of simplicity the modification referring to the phase is omitted, the *suction force* (*suction tension, suction*) *of the contents of the cell,* $S_{1_n} = 9.7$ atm. In a similar manner it is deduced that $S_{1_s} = 9.3$ atm.

The wall pressure (W), *i.e.*, the pressure which the stretched wall exerts upon the contents of the cell has the following values: At incipient plasmolysis naturally $W_g = 0.0$ atm.; in the saturation phase, $W_s = 9.3$ atm.; with the assumption that change in wall pressure is proportional to the change in volume the wall pressure in the normal phase may be calculated and shown to be $W_n = 5.4$ atm.

The turgor pressure (T), *i.e.*, the pressure which the contents of the cell exert upon the wall, is, at equilibrium, numerically equal to the wall pressure, since action and reaction are equal, but it acts in the opposite direction.

The *suction force* (*suction tension, suction*) *of the cell* (S_z) is the force per unit area with which the whole cell, consisting of the cell wall and the contents of the cell, tends to absorb water. If external forces that tend to produce tension or pressure are absent, it is composed of two forces (tensions) which operate in opposite directions: the suction force of the contents of the cell, and the wall pressure, which tends to force water from the cell. The relations may be expressed by the equation:

$$S_z = S_1 - W$$

It follows that the *suction force of the cell,* in the phase of incipient plasmolysis, in the normal phase, and in the phase of saturation, has the following values respectively:

$$S_{g_z} = S_{1_g} - W_g = 10.5 - 0.0 = 10.5 \text{ atm.}$$

$$S_{z_n} = S_{1_n} - W_n = 9.7 - 5.4 = 4.3 \text{ atm.}$$

$$S_{z_s} = S_{1_s} - W_s = 9.3 - 9.3 = 0.0 \text{ atm.}$$

Our suction force equation contains three osmotic quantities which differ in their concepts and usually also in their numerical expression. It appears from the numerical expression given above, and even more clearly from the graphic representation in figure 1, that these three quantities behave in an altogether different manner as the cell changes from the phase of incipient plasmolysis to the phase of saturation. S_1 varies but little; W increases very rapidly; and S_z decreases even more rapidly. Let particular emphasis be placed on the fact that the same cell may simultaneously possess an inner pressure of several atmospheres ($T_n = 5.4$ atm.), and nevertheless be able to take in water ($S_{z_n} = 4.3$ atm.).

Previously it was assumed that no foreign mechanical stress or strain was placed upon the cell; in this case $T = W$ numerically. If an external pressure $(+A)$ or tension $(-A)$ is present, then $T = W \pm A$, and the equation expressing the suction force takes the form: $S_z = S_i - (W \pm A)$.

Significance of the osmotic quantities

The significance of the osmotic quantities follows consequently from what has already been said.

The *suction force* (*suction tension, suction*) of the cell, $S_{z_n} = 4.3$ atm., is the quantity that is the dimensional standard for the intake, the extrusion, and the conduction of water. It is indispensable, for example, in the study of water economy.

The *suction force* (*suction tension, suction*) *of the contents of the cell,* $S_{i_n} = 9.7$ atm., must not be confounded with S_{z_n}. Thus it does not deter-

Zustand der Zelle	Grenzplasm. g	Normal n	Wassersättigung s
Volumen der Zelle (V) . .	13 209	14 122	14 779
Saugkraft des Zellinhaltes (Si) in Atm.	10,5	9,7	9,3
Wanddruck (W) in Atm.	0.0	5,4	9,3
Saugkraft der Zelle (Sz) in Atm. . . .	10,5	4,3	0,0

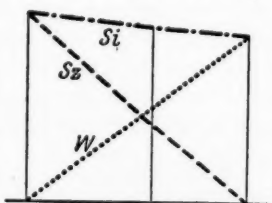

FIG. 1. Graphic representation of the osmotic quantities of a cell taken from the pith of *Impatiens*. (According to URSPRUNG 46.)

mine the amount of water absorption, but it is an important component of S_{z_n}, as is shown by the equation $S_{z_n} = S_{i_n} - W_n$. S_{i_n} is equal to the suction force of the protoplasm. It is furthermore a measure for the change in the water balance of the cell, provided the osmotic solute remains constant.

The *wall pressure,* $W_n = 5.4$ atm., naturally plays an important rôle in turgor movements. The measurement of this quantity has furthermore contributed in establishing the relations that exist between turgor pressure and growth. The rigidity of the soft-walled cell is the result of the combined action of the wall pressure and the turgor pressure. In the state of equilibrium $T = W$; when, however, asci burst open in plasmoptyse $T > W$.

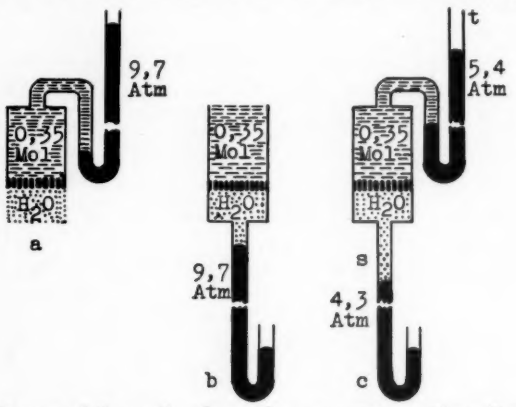

FIG. 2. Diagram of the suction force of an osmometer. (According to URSPRUNG 46.)

Turgor distention. For every change in volume, $V_s - V_g$, there is a corresponding equivalent change in turgor pressure, $T_s - T_g$, which will vary in different cells, the magnitude of which depends upon the distention. The turgor distention is the dimensional standard for the cell, in its rôle as a water reservoir, as also for turgor movements.

The *suction force* (*suction tension, suction*) *at incipient plasmolysis,* $S_{i_g} = S_{z_g} = 10.5$ atm., serves in the study of the regulation of osmotic relations (Studium der Osmoregulationen).

The *osmotic values,* O_g and O_n, are usually auxiliary quantities that are necessary in the determination of S_{i_g} and S_{i_n} of the individual cells.

II. Methods of measuring

Nothing more can be done here than to indicate the principle involved in each of the different methods and thus bring into greater relief the names employed to designate the various quantities.

1. The osmotic value at incipient plasmolysis, $O_g = 0.38$ mol cane sugar, is measured by the oldest method of measuring in this domain. It determines that concentration of a harmless non-permeable plasmolyte which at osmotic equilibrium causes the protoplasm to recede ever so little from the cell wall.

2. The *suction force* (*suction tension, suction*) *of the cell or of the cell contents at incipient plasmolysis,* $S_{z_g} = S_{i_g} = 10.5$ atm., is obtained by translating the value 0.38 mol cane sugar, obtained in method 1, into equivalent atmospheres. O_g and S_{i_g} refer, it is true, to an abnormal condition of the cell, but they nevertheless yield important results especially concerning the regulation of the osmotic relations in the normal cells.

3. The *suction force* (*suction tension, suction*) *of the contents of the cell in the normal phase,* $S_{i_n} = 9.7$ atm., may for example be determined by cryoscopy of the expressed juice. It must be remembered, however, that all methods employing press-juice yield only average values for the larger tissues or entire organs. The measurement of S_{i_n} for *individual cells* demands a knowledge of the value of O_n.

4. The *osmotic value of the contents of the cell in the normal phase,* $O_n = 0.355$ mol cane sugar, is deduced from O_g by means of the equation $O_n = O_g \frac{V_g}{V_n}$. The equivalent value in atmospheres is the value of S_{i_n}. The value of S_{i_n} cannot be determined directly from S_{i_g} by using the quotient of the volumes as a factor, because the suction force (osmotic pressure) of a solution increases at a greater rate than does the concentration.

5. The *suction force* (*suction tension, suction*) *of the cell* in the normal phase, $S_{z_n} = 4.3$ atm., is measured by determining the concentration of a harmless non-permeable plasmolyte (Osmotikum) in which the original cell volume remains constant. It is necessary that the cell wall is sufficiently distensible that the cell may show an appreciable change in volume with change of the external concentration.

6. The *wall pressure,* $W_n = 5.4$ atm., can be calculated for individual cells, in the absence of external pressure, from the equation $W_n = S_{i_n} - S_{z_n}$. The suction force of the cell, S_{z_n}, remains unintelligible so long as the wall pressure is not taken into consideration.

7. The *turgor pressure,* $T_n = 5.4$ atm., is numerically equal to the wall pressure in the absence of external forces, but it acts in the opposite direction. When external forces enter into consideration, then $T_n = W_n + A_n = S_{i_n} - S_{z_n}$.

III. Review and critique of the various terminological proposals

A. The older terminology

Before the newer methods were developed, it was customary to use either the method of incipient plasmolysis or the cryoscopic method. Sup-

pose we begin with the method of incipient plasmolysis, again taking our numerical examples from the data obtained with cells of *Impatiens*. The suction force at incipient plasmolysis was *measured,* $S_{z_g} = S_{l_g} = 10.5$ atm. The values obtained were frequently labeled as follows:

Osmotic pressure; this, however is actually only 9.7 atm.; error = 0.8 atm.

Turgor pressure; actual value is 5.4 atm.; error = 5.1 atm.

Suction force; actual value is 4.3 atm.; error = 6.2 atm.

The confusion is even greater in the following example: the author is studying water intake; he *requires to know* the suction force of the cell (4.3 atm.), but he *speaks* of turgor pressure (5.4 atm.) and he *measures* the suction force at incipient plasmolysis (10.5 atm.).

The difficulties of the cryoscopic method are similar. When this method is used to investigate water economy, *e.g.,* DIXON (**6**), HARRIS and his associates (**14–19**), KORSTIAN (**24**), the quantity determined is not the correct one. For there is no doubt that, in connection with water intake, the suction force of the cell contents does not come into consideration at all, but rather the suction force of the cell (4.3 atm.). The error of all investigations of water economy that depend on the cryoscopic method lies in neglecting to take the wall pressure into consideration.

The misunderstandings that have arisen as the result of the confusion of the osmotic quantities have been discussed elsewhere (URSPRUNG and BLUM **48**); further examples may readily be found by any one in the language with which he is familiar. The task of the new terminology must be to provide a nomenclature such that misconceptions will be avoided.

B. THE RECENT TERMINOLOGY

The quantities which are to be distinguished have been indicated in sections I and II. The individual terms will now be discussed.

INDIVIDUAL TERMS: (1) THE SUCTION FORCE OF THE CELL, $S_{z_n} = 4.3$ ATM.—As previously mentioned, the normal phase is indicated by the omission of the modification referring to the phase; consequently "suction force of the cell" is used instead of "suction force of the cell in normal phase." Of all the terms the expression "suction force" has been most objected to. Some authors criticize only the element "force" (Kraft); others reject the term "suction" as well.

(a) *Pros and cons of "suction."*—SHULL (**36**), to my knowledge the only author who has expressed himself as opposed to the term "suction" (Saugung), says: "If a careful analysis of water movement is made it will always be found that water is the active compound. The cell contents merely provide a medium of lower free water content into which water from regions of greater free water content moves. The pressure in any cell is caused by the free water entering from outside the cell. If the pressure in

a street car, when overcrowded with people who try to enter after the car is full, can be called a suction pressure, then the pressure in a cell can be called suction pressure, and the force of entry called suction tension."[4] In answer to these statements the following remarks may be permitted. So far as I know, the actual cause of the osmotic phenomenon is unknown even today (*cf.*, for example, FINDLAY **9**); consequently there is no necessity for the physiologist to restrict the terminology to any particular theory.

The ordinary mode of expression *favors* the term "suction"; then there is in use a very old expression which we find for example in suction pump, suction root, suction hair. The justification of the term suction appears most clearly if the cell is replaced by a semipermeable osmometer to which in an appropriate manner a manometer has been attached. The osmometer contains a 0.355 mol cane sugar solution (fig. 2, *c*), which has an osmotic pressure of 9.7 atm. The wall pressure, numerically equal to the turgor pressure, is compensated by the mercury pressure of 5.4 atm. in the manometer t. Of special interest to us is the osmotic quantity, which amounts to 4.3 atm. (9.7–5.4 atm.); manometer s (fig. 2) registers it, and clearly as a suction which the osmometer exerts upon the water outside. According to the data indicated on the manometer s, which are objective and independent of any particular concept of the mechanism of osmosis, we have the right to speak of a "suction" force of the cell $S_{z_n} = 4.3$ atm.

(b) *Pros and cons of "force."*—SHULL (**37**) says of suction force: "No unsuitable term should be considered immune from change, especially one not over 15 years old, just because it has been commonly employed in Europe." This assertion concerning the age of the contested term does not by any means agree with the facts. Incidentally PFEFFER (**32**, p. 77) speaks of "suction force," but particularly DEVRIES (**51**, p. 561); since that time the term is found in the literature of plant physiology in ever so many languages [*e.g.*, NOLL (**42**) in STRASBURGER'S Lehrbuch, p. 161; HABERLANDT (**13**, p. 351); FITTING (**10**, p. 209); VINES (**50**, p. 429); EWART (**8**, p. 77).] Consequently the term "suction force" is about as old as the history of osmotic relations in cells. Since it did not seem to me absolutely necessary to coin a new term, while developing mensural methods in 1916, I employed the term already in use. That the term is not strictly correct in the physical sense, I have already in 1916 emphatically pointed out (URSPRUNG and BLUM **47**, p. 529) as follows: "With reference to the terminology, the term "force" should be retained, even though it is a matter of $\frac{\text{force}}{\text{area}}$, therefore a quantity not mensurable in kilograms, but in atmospheres."

[4] SHULL (**38**) also says in a later paper: "If the expansive force exerted in the interior of an automobile tire when we force air into it can legitimately be called a 'suction force,' then also we can call the expansive force of a cell when water is forcing its way into the cell a 'suction force.'"

(c) *Suggested substitutes for "force."*—Suction "pressure"—as suggested by STILES (**40, 41**), and BRIEGER (**4**), a student with RENNER—has the correct dimensions but is a physical impossibility, since a manometer can register either suction or pressure, but not both at the same time (URSPRUNG **45**, p. 196). The expression appears to have been abandoned, at least in German-speaking countries (RENNER **35**, p. 749; GRADMANN **12**, p. 635).

Suction "value" (GRADMANN **11, 12**, p. 636), suction "ability" (PRINGSHEIM **33**, p. 749), suction "potential" (OPPENHEIMER **30**, p. 131; **31**, p. 526) are terms that indicate sufficiently that there is a question of *capacity* which does not by any means always lead to water intake. This I had already said in 1916 (URSPRUNG and BLUM **47**, p. 530) and still more clearly in 1920 (URSPRUNG and BLUM **48**, p. 202) with the definition: Suction force of the cell = the force with which the cell *strives* to take in water. Of course it is evident that the cell cannot take in water if it does not suck more forcibly than the surrounding medium. I did not deem it necessary to emphasize that fact by applying a special term. One does not speak of "Pferdepotential" but of "Pfedekraft" even though the horse will pull the wagon only if the load is not too heavy.

Suction "tension" (SHULL **36**, p. 214; BECK **1**, p. 425) has the correct dimensions, and is physically more nearly correct than suction "force" (*cf.* also URSPRUNG **45**). If a new term had to be chosen, one would choose tension (Spannung); but since the term suction "force" is already a half century old and has given rise to no misconceptions, it may as well, as I see it, be retained in the future. Even the physicists tolerate a similar liberty in the use of terms, *e.g.*, using "Dampfspannung" instead of "Spannkraft" of steam (CHWOLSON **5**, p. 709); but if Spann"kraft" is permitted in physics, why should the term Saug"kraft" be forbidden in physiology? Furthermore, so long as we retain such terms as cryptogams, tracheae, and leucoplasts one has no right to forbid suction force. It is after all simply a matter of taste whether one, with deference to the historical development, continues to use an old term which has given rise to no misconceptions, or whether one prefers a faultlessly correct expression.

(d) *Counter suggestions for "suction force."*—"Suction" (SIERP in STRASSBURGER'S Lehrbuch, 18th ed., p. 76) is a short and clear term but appears to have greater difficulty in becoming established than does suction force or suction tension.

"Water absorbing power" (THODAY **43**, p. 110) does not have the correct dimensions, and is rather lengthy.

"Pouvoir osmotique" (LECLERC DU SABLON **25**, p. 24) reminds one of PFEFFER'S "osmotische Triebkraft" (**32**, pp. 76, 84) but it does not indicate whether it signifies suction or pressure.

Concerning the terms "Hydratur" and "Wasserzustand" the reader is referred to section (2) later.

"Turgor deficit" employed by CURTIS (private communication from BECK), it seems to me, can mean only one thing, *i.e.*, a difference between the maximum turgor pressure which is possible and the pressure which actually exists. As is shown in figure 1, the turgor deficit of our *Impatiens* cell is 9.3 – 5.4 = 3.9 atm., while the suction force of the cell is $S_{z_n} = 4.3$ atm. Turgor deficit and suction force of the cell are not equal numerically, nor are the terms conceptually alike; they cannot therefore be used synonymously.

SHULL (**37**) "sees no good reason for using any other term than osmotic pressure in connection with the turgidity of plant cells." But how, then, shall our osmotic quantity 4.3 atmospheres be designated? The "osmotic pressure" is really 9.7 atm., the "turgor pressure" 5.4 atm.; accordingly the difference, if we are to preserve both these terms, must be designated as "the difference between the osmotic pressure and the turgor-pressure," a term surely too detailed to find any support.[5]

"Traction" was suggested (LIVINGSTON, private communication to BECK, 1927) presumably to replace tension, but I have no further information concerning it.[6]

(e) *Other suggestions.*—Distinctions have been made between static and kinetic, between potential and actual (OPPENHEIMER **30, 31**; RENNER **35**), between absolute and relative suction force (HUBER **22**; BENEKE-JOST **3**, vol. I, p. 57), and between osmotic and swelling (imbibitional) suction force.

In water intake by the cell osmotic and swelling forces may be associated; the former are usually determinative. Both components in a state of equilibrium must of course be equal to one another: a change in one necessarily causes a corresponding change in the other.

The *Impatiens* cell (fig. 1) with its suction force, $S_{z_n} = 4.3$ atm., can take in water, exude water, or be in equilibrium with the environment. Which case actually obtains depends upon the value and the direction of the suction force gradient (Saugkraftgefälle) of the environment of the cell, as well as upon the resistance to transfusion (Filtrationswiderstand). Just as one in physics speaks of kinetic and static, of actual and potential energy, according to whether or not it results in motion, so one may of course do the same thing in physiology.

[5] SHULL has suggested "net osmotic pressure" for this quantity.

[6] Translator's note: The author refers to a letter from LIVINGSTON to the translator January 3, 1927, in which LIVINGSTON suggested in an informal way a number of terms which were submitted by the translator, of his own accord, to URSPRUNG. The discussion is too lengthy to be given here.

The only question is whether, to avoid misunderstanding, it is necessary to speak of a static suction force when the osmotic energy remains potential, and of a kinetic suction force when the osmotic energy becomes actual and sets the water in motion. An example from mechanics may serve to elucidate this. Suppose that two similar, one-horsepower tractors are attached to the same vehicle; in one case the tractors pull in the same direction and in the other they pull in opposite directions. It is quite unnecessary to refer in the first case to kinetic or actual horsepower and in the second to static or potential horsepower; similarly it is altogether superfluous to make a like distinction in physiology.

Moreover, the expressions *absolute* and *relative* suction force which are employed to signify that the cell with a suction force of 4.3 atm. is in the first case sucking against pure water, and in the second case against an osmotically active environment, are to say the least superfluous. As has already been shown (URSPRUNG and BLUM **49**, p. 2), the suction force is the same in both cases; the *suction force gradient,* however, is different in the environment of the cell. This fact is simply and unequivocally expressed by the use of this old term (suction force).

New and unnecessary terms should, in my opinion, be avoided, as they are a useless complication of nomenclature which unnecessarily makes understanding difficult for the beginner and layman.

To summarize, then, for the quantity $S_{z_n} = 4.3$ atm. the following terms are recommended: suction force of the cell, suction tension of the cell, or suction of the cell; in addition one may use the expressions suction force gradient, suction tension gradient, or suction gradient.

(2) SUCTION FORCE OF THE CELL CONTENTS, $S_{l_n} = 9.7$ ATM.—What has just been said regarding suction force of the cell applies as well to the discussion of the cell contents. We may therefore give our attention immediately to the counter suggestions.

(a) *Counter suggestions.*—Osmotic pressure is an excellent term in the field of physics, which, however, has not proved adequate in plant physiology. Before 1916 all of the osmotic quantities measured or used were simply referred to as osmotic pressure, even when they differed in numerical value as well as in their concepts. For example, previous to that time osmotic pressure was sometimes understood to mean $S_{z_n} = 4.3$ atm., sometimes it meant $S_{l_n} = 9.7$ atm., sometimes $T_n = 5.4$ atm., sometimes $S_{z_g} = 10.5$ atm. That such a state of affairs must lead to serious confusion is self-evident.

If the physiologist wishes to employ the term "osmotic pressure" he must use it in the sense of the physicist, *i.e.*, the maximum pressure which the cell sap can sustain in an osmometer which is provided with a semi-

permeable membrane. Accordingly it is correct to say that the "osmotic pressure" of the cell sap is 9.7 atm. Let us now examine this term and see whether it serves the physiologist's purpose.

Referring to figure 2, *b*, note that the 0.355 mol cane sugar solution exercises a *suction* upon the water which lies on the opposite side of the membrane and above the mercury, which amounts to 9.7 atm. In figure 2, *a*, the manometer shows a *pressure* of 9.7 atm. The 9.7 atm. may be regarded as a tension as well as a pressure. The cell which we have been considering possesses a turgor pressure of 5.4 atm. as well as an osmotic pressure of 9.7 atm.; since both pressures are the immediate result of the osmotic phenomenon, they may, though distinct, readily be confounded. Let a recent case serve as illustration. (For less recent cases *cf.* URSPRUNG and BLUM **48**.) WENT (**55**) says: "The osmotic pressure of the contents of the cell is received by the stretched cell wall." "Osmotic pressure" is spoken of, which in the case of our illustrative cell is 9.7 atm., while the author has in mind turgor pressure which is really 5.4 atm.

Since the 9.7 atm. (refer to equation $S_{z_n} = S_{i_n} - W_n$) tend *to carry the water into the cell* and the 5.4 atm. tend *to force water from the cell*, it does not seem desirable to apply the same term "pressure" indiscriminately in both cases. Since there is no opposition to the term "turgor pressure" as here used, there remains nothing else but to drop the expression "osmotic pressure" if we are to avoid being misunderstood.

"Osmotic value" was suggested as a substitute for "suction force of the contents of the cell" by HÖFLER (**21**) and WALTER (**53**). The latter desires that the osmotic value should be expressed only in atmospheres, while I express it in molal units.

Under the caption "value" anything may be understood *a priori*. If, however, we are to give unequivocal expression to what we mean, we should agree upon one mode of expression of the quantity (*cf.* also sec. 4). WALTER'S suggestion, to express the value only in atmospheres, is not practicable; first because we frequently need the molal expression (*e.g.*, in the equation $O_n = O_g \frac{V_g}{V_n}$, which is necessary for the determination of S_{i_n} of the individual cell), and then because it is not always possible to translate the molal value into atmospheres partly because the concentration data are insufficient. As we already have the expression "suction force (suction tension, suction) of the contents of the cell," the simplest course would be to continue to express the "osmotic value" in molal units.

"Osmotic concentration" has been used for a long time by HARRIS and his associates (**14–19**). Recently PRINGSHEIM (**33**) also has suggested the term as a substitute for "osmotic pressure." DIXON and ATKINS (**7**) as also KORSTIAN (**24**) speak of "sap concentration." These designations

are not to be recommended as substitutes for the quantity S_i, which is here in question, for it must be measured in atmospheres (as follows consequentially from the equation $S_z = S_i - W$). Logically a concentration should not be expressed in atmospheres.

"Density of cell sap" is used by KORSTIAN (**24**) as synonymous with "osmotic pressure." This expression also is unsuitable since density cannot be measured in atmospheres.

"Water relations," "water conditions," and "Hydratur" are expressions that WALTER (**52–54**) suggests as substitutes for "suction force (suction tension, suction) of the contents of the cell" and he expresses the quantity in atmospheres. He uses three methods for the measurement of "Hydratur" which yield results that are numerically different and are altogether different in concept: (1) Cryoscopy of the expressed sap which gives an average value of $S_{i_n} = 9.7$ atm.; (2) Determination of the vapor pressure of intact cells which gives values of $S_{z_n} = 4.3$ atm. and not values for S_{i_n}; (3) The method of incipient plasmolysis by which $S_{i_g} = S_{z_g} = 10.5$ atm. is determined. Not only do these methods yield quantities that are altogether different in themselves but they are rechristened and defined in a manner that introduces confusion. While WALTER thinks he is measuring the "water condition" or "Hydratur," usually by determining the quantity $S_{i_n} = 9.7$ atm., RENNER (**35**) explains that the "water condition" or "Hydratur" can agree in value only with the suction force of the cell $S_{z_n} = 4.3$ atm. This confusion follows from the fact that both authors are measuring different quantities and apply common names indiscriminately. WALTER studies the situation from the point of view of the suction force of the protoplasm, while RENNER regards it from the point of view of the suction force of the cell. These recent examples show how futile it is to form a new, indefinite and unnecessary terminology; it makes things very difficult for those who are not very familiar with the subject, while nothing of value is gained. I retained, whenever it was at all possible, the already-existing terms, precisely for the purpose of not overburdening the nomenclature.

(3) THE SUCTION FORCE AT INCIPIENT PLASMOLYSIS, $S_{i_g} = S_{z_g} = 10.5$ ATM.—Here again that which was already mentioned about "suction force" holds, *i.e.*, that "suction tension" or "suction" should be considered synonymous with it. The modifications "of the cell" and "of the contents of the cell" become superfluous, since $S_{i_g} = S_{z_g} = 10.5$ atm. At this time it is probably self-evident that only the suction force at incipient plasmolysis can be measured and not all other kinds of quantities. It follows that previous misunderstandings in so far as they arose from confusion of terms should henceforth be eliminated. The determination of $S_{i_g} = 10.5$ atm. by the plasmolytic method has rendered physiology great service (one has only to

recall the work of DE VRIES), and will continue to do so in the future (*e.g.*, in the study of osmotic regulation) if one only uses a proper plasmolytic agent, and if care is taken to interpret the results correctly.

(4) THE OSMOTIC VALUE, $O_n = 0.355$ MOL CANE SUGAR.—

(5) THE OSMOTIC VALUE AT INCIPIENT PLASMOLYSIS OR INCIPIENT PLASMOLYSIS VALUE, $O_g = 0.38$ MOL CANE SUGAR.—

The term "osmotic value" (when the attribute is lacking, "in the normal phase" is understood) signifies the molal expression of the concentration of the plasmolyte which is isotonic with the cell sap, when the cell has the normal volume (URSPRUNG and BLUM **48**; BECK **2**). The osmotic value at incipient plasmolysis, $O_n = 0.38$ mol cane sugar, must first be determined before the quantity $O_n = 0.35$ can be deduced from it. The deduction is made by means of the equation, $O_n = O_n \frac{V_g}{V_n} = 0.355$ mol cane sugar, in which V_g is the volume of the cell at incipient plasmolysis, and V_n the volume in the normal phase.

(a) *Other suggestions.*—HÖFLER (**21**) made the suggestion which was seconded by HUBER (**23**) that the osmotic value should be expressed in atmospheres as well as in molal units. According to this suggestion we should write: $O_n = 0.355$ mol cane sugar $= 9.7$ atm.

As previously mentioned, the term value may, *a priori*, be variously employed, but in the interest of a simple nomenclature, which admits of but one interpretation, we should agree upon some simple but adequate term. Furthermore, a 0.355 molal cane sugar solution can only be equivalent to but not identical with a pressure of 9.7 atm. Different quantities measured in different systems of units may not be given a common name just because they have equivalent values. Now if the quantity measured in atmospheres be designated as the suction force (suction tension, suction) of the contents of the cell, there is nothing to prevent us from calling the quantity with its equivalent values, expressed in molal concentration units, the osmotic value.

Osmotic concentration.—PRINGSHEIM (**33**) suggests that the term "osmotic value" be rejected as not sufficiently definite, while WALTER (**53**, p. 83) wishes to have the quantity introduced even into the suction-force equation. Let it be recalled that just as in the case of the term suction force, we are dealing with a *time-honored* term that for long has been employed in plant physiology. It harmonizes very well, too, with the ordinary modes of speech; for just as we speak of the monetary value of various things we may discuss the osmotic value of different cell saps. Accordingly it is certainly permissible to speak of the "osmotic value" as being 1 mol cane sugar solution. The substitute which PRINGSHEIM (**33**) proposes, "osmotic concentration" of 1 mol cane sugar solution, is decidedly not an improvement. In the first place the new term may give the impression that

the cell sap contains a 1 mol cane sugar solution; then again the concentration is measured in mols but not necessarily with cane sugar; finally the concentration is not the only factor to be considered in the osmotic phenomena for the chemical constitution of the sap is a factor too.

(6) THE WALL PRESSURE $W_n = 5.4$ atm.—

(7) THE TURGOR PRESSURE $T_n = 5.4$ atm.—

From the nature of things it follows that the wall pressure is the pressure that is exerted by the wall upon the contents of the cell. Turgor pressure is generally admitted to be the pressure exerted by the contents of the cell upon the wall. In a condition of equilibrium $T = W$, when no foreign forces enter the consideration. If foreign forces are to be considered, $T = W \pm A$. I am not aware of any terms that may have been suggested as substitutes for these.

(b) *The suction-force (suction tension) equation.*—My equation reads: The suction force of the cell = the suction force of the contents of the cell – the wall pressure; $S_z = S_l - W$; of course suction force may be replaced by suction tension or by suction. If foreign forces enter into consideration the equation becomes: $S_z = S_l - W \pm A$. T is given by $W \pm A$.

Suggested substitutes.—RENNER (**34**): Saugkraft der Zelle = osmotische Druck – Turgordruck.

$$S = P - T.$$

This equation was published before mine; both were established independently.

THODAY (**43**): Water absorbing power = osmotic pressure – turgor pressure.

$$p = P - T.$$

HÖFLER (**21**): Saugkraft der Zelle = osmotischer Wert (in Atm.) – Turgordruck.

$$S = O - T.$$

LECLERC DU SABLON (**25**) pouvoir osmotique du liquide exterieur = pouvoir osmotique du suc cellulaire – turgescence.

STILES (**40, 41**): suction pressure = osmotic pressure – wall pressure.

$$S = P - T.$$

WALTER (**53**): Saugkraft = osmotischer Wert (in Atm.) – Wanddruck.

SIERP (**39**): Saugung der Zelle = Saugung des Zellinhaltes – Wanddruck.

Several of the suggested substitutes place turgor pressure in the place of wall pressure in my equation; I do not recommend this form of writing the equation as it does not indicate the actual condition. The quantity which acts counter to the intake of the water is not the turgor pressure but the wall pressure (ev. $W \pm A$).

Other variations often consist in replacing S_l by the osmotic pressure. As was shown previously (2) this is not to be recommended; first because it leads to confusion with the turgor pressure, and then again because it is to no good purpose to use the common term "pressure" for a quantity which tends to press water from the cell and another which tends to draw water into the cell.

The why and the wherefore of expressing the osmotic value in molal units rather than in atmospheres has been discussed (*cf.* sections 2, 4, 5).

If suction tension or suction is to be preferred to suction force it is a matter of taste so far as I am concerned.

(c) *Suggestions on the reduction of the number of terms.*—As was mentioned above, SHULL (**36–38**) prefers to use no term other than "osmotic pressure" in connection with the turgidity of plant cells. Similarly LUBIMENKO (**27**) speaks only of "pression osmotique" and "pression de turgescence."

HÖBER (**20**) speaks of "osmotischen Druck" and "Turgor." OLTMANNS (**29**) even tries to get along with the term "turgor" alone.

In itself, of course, the notion of reducing the number of terms is very welcome; but for all that, the basic purpose, *i.e.*, the possibility of clear expression of ideas and the elimination of misunderstandings, must not be sacrificed. That two terms will not suffice to express unequivocally seven quantities will probably not be questioned in view of the illustrations which were drawn from various authors, and further demonstration will hardly be necessary. Referring again to SHULL'S reviews, he wrote (**36**) in reference to BLUM'S measurements of suction force in alpine plants: "These suction force studies show in a different way by plasmolytic means, just what HARRIS'S studies of freezing point depressions have shown, a general correspondence of plant cells to the conditions of the habitat." If he intends to convey the notion that the same quantity was measured in two different ways, he is in error.[7] HARRIS—referring once again to the illustrative cell of *Impatiens*—measured $S_{l_n} = 9.7$ atm.; BLUM measured $S_{z_n} = 4.3$ atm. The fact is that the two quantities usually do vary in the same sense. Thus while passing from the phase of incipient plasmolysis to the saturation phase (*cf.* fig. 1), both experience a decrement, but it is slight in S_l, *i.e.*, from 10.5 to 9.3 atm., and considerable in S_z, *i.e.*, from 10.5 to 0.0 atm. If the quantity S_{l_n} is determined in the study of the water economy, faulty individual values are obtained, because the real indicator of the conditions is S_{z_n}.

Whoever attempts to carry on with only one or two terms is bound to fall into the same errors that were committed in the past, *i.e.*, labeling indiscriminately $S_{z_n} = 4.3$ atm., $T_n = 5.4$ atm., $S_z = 10.5$ atm., whichever quantity is desired, as "osmotic pressure."

[7] He had no such intention.

Summary

The earlier studies of osmosis in plants led to confusion because a common name was applied to different quantities and because of attempts to measure these quantities by a common method. The creation of methods which permit the determination of the different quantities numerically as well as in concept, constitutes the essential difference between the more recent studies and the older ones. In order to avoid misunderstandings a new terminology became necessary. It embraces the expressions: Suction force (suction tension, suction) of the cell, S_{z_n}; suction force (suction tension, suction) gradient; suction force (suction tension, suction) of the contents of the cell, S_{l_n}; suction force (suction tension, suction) at incipient plasmolysis $S_{z_g} = S_{l_g}$; osmotic value, O_n; osmotic value at incipient plasmolysis = incipient plasmolysis value, O_g; wall pressure, W_n; turgor pressure, T_n; and the turgor distention produced by the turgor pressure. The terms are, as they should be, unequivocal, simple, and easy to understand. In so far as it was possible, they were linked to the old terminology. The terms suction and pressure were chosen in accord with the indications on the manometer. Wherever the manometer permits both designations, the form that seems best adapted to avoid misunderstandings has been chosen. The prime purpose of terminology is simplicity and the elimination of error. When these ends can be obtained in different ways a certain flexibility in the mode of expression should be tolerated. With me it is a matter of taste whether suction tension or suction be considered synonymous with suction force. Even though it is desirable to use but a single term for a given quantity, it is not absolutely necessary, so long as the essential purpose of terminology is not defeated. For decades the significance of terminology was underestimated, but we need not for all that go to the other extreme.

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DETERMINATION OF AMINO NITROGEN IN PLANT EXTRACTS

NEIL W. STUART

Introduction

In nitrogen partition studies it is customary to estimate the amount of nitrogen contained in the alpha-amino groups of amino acids by means of the Van Slyke gasometric or the Sörensen formol titration method. As is generally known, these methods were developed for the analysis of pure amino acids or products of protein hydrolysis as applied particularly to animal tissues. Since plant extracts usually contain water soluble pigments and buffering substances which interfere with the formol titration, the Van Slyke method is more frequently employed.

While engaged in some nitrogen partition studies with alcoholic extracts of apple leaves (**9**), the writer observed that the yield of amino nitrogen by the standard Van Slyke procedure was in excess of the total soluble, non-protein nitrogen. This finding has repeatedly been confirmed and the same error noted in varying degrees in several other plant tissues extracted with water or 80 per cent. alcohol. The present investigation was undertaken to study the factors responsible for these anomalous results, and to develop, if possible, corrective measures.

Materials and methods

Plant tissues used in this study included: apple leaves; rhubarb petioles and leaves; clover roots and tops; young cabbage; tomato, soy bean, and sunflower plants; begonia petioles and leaves; and potato tubers.

ALCOHOLIC EXTRACTION OF SOLUBLE NITROGENOUS SUBSTANCES.—Representative 100-gm. samples of tissue were preserved in sufficient hot 95 per cent. alcohol to give a final concentration of 80 per cent. The alcohol was filtered off and the residue extracted eight times with 80 per cent. alcohol by the decantation method (**9**).

WATER EXTRACTION OF SOLUBLE NITROGENOUS SUBSTANCES.—Similar samples of fresh tissue were passed through a Nixtamal mill and weighed portions of the pulp were ground in a mortar with nitrogen-free sand and water at a temperature of 25° C. The soluble material was expressed by hand through finely woven cloth. The residue was again ground in the mortar and the process repeated until complete extraction of the soluble, non-protein nitrogenous substances was obtained.

Analytical procedure

TOTAL NON-PROTEIN NITROGEN.—Total nitrogen of the protein-free extracts was determined by the Kjeldahl method. When it was possible to

demonstrate the presence of nitrates by the diphenylamine test, the modified method of PUCHER *et al.* (**6**) was used.

ALPHA-AMINO NITROGEN.—All amino determinations were made with the Van Slyke micro-apparatus, using 2- or 4-ml. aliquots. Blank determinations and the proper corrections for temperature and pressure were made regularly. Deaminization was allowed to proceed for a period of five minutes and the usual precautions to insure accurate comparable results were consistently observed. Values reported for amino nitrogen are averages of duplicate determinations which agreed closely.

Difficulty was encountered in obtaining a satisfactory anti-frothing reagent for use during deaminization. Several samples of caprylic alcohol proved unsatisfactory owing to the formation of a large blank. A number of other materials were tested in blank determinations and with plant extracts. Of these toluene proved the best. When used in 0.25-ml. portions the troublesome frothing was largely suppressed.

Experimental results

SUBSTANCES IN PLANT EXTRACTS CAUSING ERRORS IN THE VAN SLYKE METHOD

AMMONIA.—It is well known that ammonia reacts with nitrous acid in the amino determination, liberating free nitrogen. VAN SLYKE reported (**13**) that 36.3 per cent. of the ammonia contained in an M/5 solution of ammonium sulphate was liberated in five minutes by nitrous acid in the macro-apparatus. It seemed desirable to repeat this experiment under the conditions encountered in this study. Solutions containing 1.0, 0.5, 0.25, and 0.125 mg. of ammonia per ml. were prepared from ammonium sulphate and 4-ml. aliquots analyzed in the micro-apparatus. It was established that the yield of nitrogen was a linear function of the concentration of ammonia and approximately constant under a given set of conditions. Thus in one set of experiments, conducted at a temperature of 30° C. and barometric pressure of 765 mm., an average of 35.2 per cent. of the ammonia was obtained as amino nitrogen following a 5-minute deaminization period. This percentage recovery was found to vary from time to time, being influenced apparently by temperature, time, rate of deaminization, etc. Accordingly it is doubtful whether attempts to apply a corrective factor for the ammonia present in solutions analyzed will prove uniformly satisfactory. Hence the removal of free ammonia, if present in appreciable quantities, is necessary for accurate amino determination.

It is generally considered that only small amounts of ammonia are found in plant tissues. In certain acid plants, however, as the begonia and rhubarb, ammonia may constitute a large proportion of the total soluble, non-protein nitrogen. Obviously any amino determinations made on these extracts would be greatly in error if the ammonia were not previously re-

moved. It is of interest to note that CULPEPPER and CALDWELL (3), who have recently studied the nitrogen metabolism of rhubarb in some detail, encountered difficulty in interpreting the results of their amino determinations. These investigators attributed the irregularities and fluctuations of their data to the effect of preservation and storage of the tissue in alcohol. While this is undoubtedly a factor, the ammonia content of the tissues, which apparently was not considered, would vitiate the results of the amino determinations if the rhubarb used in the present study was at all comparable.

Frequently plants grown under disturbed nutritional conditions are used for analytical studies. Under such conditions ammonia accumulation often occurs as a result of a breakdown in the nitrogen metabolism. Ammonia may also increase in stored alcoholic extracts of plant tissues, according to WEBSTER (15). For these reasons some investigators, as THOMAS (11), CHIBNALL (2), NIGHTINGALE (4), and others, have employed methods whereby the ammonia is removed prior to the amino determination. This may be accomplished by a preliminary distillation to remove the free ammonia. In some instances the amide linkages are hydrolyzed and the total ammonia is then removed prior to making the amino determinations. This procedure measures all of the free alpha-amino groups, but some hydrolysis of peptide linkages may have occurred during the amide hydrolysis. Frequently the filtrate from the phosphotungstic acid precipitate of basic nitrogen is analyzed for amino nitrogen. This filtrate contains only the mono-amino acids and simple peptides.

DERIVATIVES OF PHENOLS.—Investigation disclosed that the di- and trihydric phenols and their acid derivatives (as the tannins) were readily oxidized in the Van Slyke determination with the formation of gases difficultly soluble in the alkaline permanganate. The amount of this gas decreased somewhat upon long continued contact with the permanganate. The amount of gas formed seemed to be correlated with the number and position of the hydroxyl groups present in the compound. Thus the trihydric phenol, phloroglucinol, yields considerably more gas than the dihydric resorcinol. When the hydroxyl groups are present in the ortho position, as in catechol or pyrogallol, much less of the insoluble gas is produced. Intermediate values were obtained for hydroquinone which has the para grouping of the hydroxyls. Ordinary phenol has little effect. It would therefore be expected that the compounds which occur in plants as esters and glucosides would react with nitrous acid according to the type of linkage, number of free hydroxyl groups, orientation of these groups, and probably other factors which are still obscure.

The tannins, which may be regarded as phenol acids, or glucosides of these acids, are widely distributed in plant tissues. Pure tannic acid

(Merck's) reacts readily with nitrous acid, forming a gas which would be measured as nitrogen in the amino determination. Extracts of oak galls known to contain large quantities of tannin were also found to react in the same manner. Many other so-called secondary plant substances are known to contain phenolic groups. Such a compound, for instance, is the glucoside phlorizin in which phloroglucinol occurs as an ester. This material is known to be present in apple tissue in large quantities. Pure phlorizin was found to react in the amino determination with the formation of small quantities of insoluble gas.

MISCELLANEOUS SUBSTANCES.—A further source of error lies in the compounds extracted with 80 per cent. alcohol but insoluble in water. When these compounds, consisting of pigments, lipides, and other material of indefinite composition, are suspended in water and introduced into the Van Slyke apparatus, a variable amount of gas insoluble in the alkaline permanganate is obtained. It is, however, a simple matter to remove these materials by the addition of a small amount of toluene or chloroform to the concentrated water suspension remaining after the removal of the alcohol. The writer prefers chloroform since it sinks to the bottom of the flask, carrying the lipides with it and permitting the clear supernatant extract to be withdrawn. The possibility must be kept in mind that other chemical groups may react with nitrous acid, reducing it to nitrogen gas or liberating other insoluble gases. Tangible evidence for this suggestion is afforded by the well known fact that ethyl alcohol and acetone form large quantities of gas when subjected to the conditions of the amino determination.

PROCEDURES TO REDUCE ERRORS IN THE VAN SLYKE METHOD WHEN APPLIED TO PLANT EXTRACTS

In order to discover the most satisfactory method to reduce errors in the Van Slyke method when applied to plant extracts, several plant tissues were extracted with water and with 80 per cent. alcohol, and the extracts were subjected to different treatments prior to the determination of amino nitrogen in the extracts. The soluble proteins were removed from the water extracts with colloidal ferric hydroxide as described by THOMAS (**10**). The pigments, lipides, etc., were removed from the alcoholic extracts by adding chloroform to the alcohol-free filtrates. The emulsion was flocculated with magnesium sulphate as previously described (**9**).[1] Suitable aliquots of these partially cleared water and alcoholic extracts were then subjected to further treatments.

[1] This method was originally suggested by Dr. T. G. PHILLIPS of the University of New Hampshire.

PRECIPITATION WITH NEUTRAL LEAD ACETATE SOLUTION.—One lot of aliquots was further cleared with saturated neutral lead acetate. The excess lead was removed with anhydrous sodium oxalate.

ADSORPTION WITH DECOLORIZING CARBON.—A second lot of aliquots was stirred at intervals with decolorizing carbon for one hour, filtered, washed, and the filtrates concentrated. The degree of adsorption is admittedly indefinite and probably depends upon the concentration of adsorbent and extract and the time. As far as possible these factors were kept constant.

ADSORPTION WITH CALCIUM OXIDE.—To a third lot of aliquots sufficient calcium oxide was added to make the extract slightly alkaline. The mixture was stirred at intervals for one hour, filtered, washed, and the filtrate concentrated after making faintly acidic with acetic acid.

DISTILLATION WITH SOLID CALCIUM OXIDE.—Distillation of a fourth lot of aliquots with solid calcium oxide under reduced pressure for one hour at 40°–45° C. was carried out in the usual VAN SLYKE ammonia apparatus (**12**). A slight excess of calcium oxide was used as determined by preliminary titration of a small portion of the extract. The extract was filtered from the calcium, washed, acidified with acetic acid, and concentrated. The ammonia was collected in 0.02 N acid and titrated to a methylene blue-methyl red indicator with 0.02 N base. This method was developed by PLIMMER and ROSEDALE (**5**) to permit controlled alkalinity in the determination of ammonia by the Van Slyke method. The same method was later applied to extracts of apple wood and leaves by THOMAS (**11**), who noted that this method, besides removing the ammonia, adsorbed the materials which cause frothing during deaminization. The results of the present study have fully confirmed the findings of these investigators, and in addition have shown that this distillation results in a decrease in the amount of gas measured as amino nitrogen. The results of these preliminary analyses are shown in tables I and II.

The data in tables I and II indicate that all treatments resulted in a decrease in amino nitrogen as compared with the direct determination on the extracts which were only partially clarified in removing the alcohol, lipides, protein, etc. This decrease was fairly uniform for a given tissue when treated with the precipitating and adsorbing agents, but in no instance was it so great as that obtained by distillation. The latter method was accordingly selected for further study. The pronounced difference between the amounts of nitrogen extracted by water and by alcohol is typical of many analyses and will be discussed in a later section of this paper.

The possibility must be kept in mind that the distillation procedure as previously described may result in a loss of amino acids through occlusion or other means, thereby accounting for a portion of the reduction in yield. Since there are no methods for determining the absolute amount of any

TABLE I

AMINO NITROGEN IN WATER EXTRACTS OF PLANT TISSUES AFTER DIFFERENT TREATMENTS OF THE EXTRACTS

TREATMENT OF EXTRACTS	WATER EXTRACTS OF 100 GM. OF FRESH TISSUE		
	APPLE LEAVES	TOMATO PLANTS	POTATO TUBERS
	AMINO N	AMINO N	AMINO N
	mg.	*mg.*	*mg.*
Colloidal ferric hydroxide to remove soluble proteins	80.3	38.5	148.0
Further treatments of protein free extracts:			
Neutral lead acetate	34.1	33.1	143.3
Carbon	30.7	33.3	140.7
Calcium oxide	33.5	33.4	137.6
Distillation with calcium oxide	22.4	30.5	123.8

TABLE II

AMINO NITROGEN IN 80 PER CENT. ALCOHOLIC EXTRACTS OF PLANT TISSUES AFTER DIFFERENT TREATMENTS OF THE ALCOHOL-FREED EXTRACTS

TREATMENT OF EXTRACTS	ALCOHOLIC EXTRACTS OF 100 GM. OF FRESH TISSUE		
	APPLE LEAVES	TOMATO PLANTS	POTATO TUBERS
	AMINO N	AMINO N	AMINO N
	mg.	*mg.*	*mg.*
Chloroform to remove pigments, lipides	44.0	27.7	83.5
Further treatments of pigment and lipide free extracts:			
Neutral lead acetate	19.5	23.2	78.2
Carbon	18.5	21.8	76.0
Calcium oxide	20.7	21.6	78.2
Distillation with calcium oxide	14.8	20.4	70.2

plant constituent, this question cannot be definitely answered. However, numerous tests with the pure amino acids, leucine and arginine, and the half-amide of aspartic acid, asparagine, resulted in quantitative recovery after the distillation. Pure amino acids could also be quantitatively re-

covered when added to plant extracts which had previously been distilled with calcium oxide and analyzed for amino content. Furthermore, when the amino acid solutions were added to the partially clarified but undistilled plant extracts, the theoretical value could be obtained by analysis after distillation. Their value was determined by distilling other aliquots of the same extract without addition of amino acids and noting the decrease in amino value.

Quadruplicate determinations on individual extracts were found to agree closely. Certain extracts were redistilled and it was established that no further decrease occurred after the first treatment. On the basis of these studies it is concluded that treatment with calcium oxide has no effect on the amino acids contained in plant extracts. The decrease in yield must then be due to other factors.

The calcium oxide residue remaining after distillation was subjected to study. It was found possible to remove the adsorbed material with acetic acid, and after neutralization it could be analyzed directly. Small amounts of gas measured as nitrogen in the Van Slyke determination were obtained which varied with different extracts. The amounts, however, could not account for the magnitude of the reduction in amino nitrogen brought about by distillation. The distillation must have exercised some denaturing effect on the interfering substances. Aside from precipitating or occluding certain materials on the surface of the calcium oxide, an oxidizing effect should be obtained as well, since a stream of air is drawn through the suspension. Under such conditions it was observed that tannic acid as well as the tannin from oak galls could be quantitatively precipitated or denatured so that no gas insoluble in alkaline permanganate was liberated in the amino determination. Distillation with calcium oxide was therefore considered to be the best method since its use removes the free ammonia, adsorbs the materials which cause frothing during deaminization, and precipitates or denatures the tannins.

After this investigation had been completed the work of RAHN (7) came to the writer's attention. This worker noted that when plant extracts were treated with tannic acid to precipitate the soluble protein, difficulty was encountered in making the amino determination by the Van Slyke method. The difficulty was attributed to a reduction of nitrogen trioxide, N_2O_3, said to arise in the determination, to elemental nitrogen. It is generally considered that nitric oxide, NO, rather than N_2O_3, arises in the determination. The latter compound is formed only at low temperatures from NO and NO_2 and at room temperature would not exist. RAHN reported that the tannic acid could be satisfactorily removed by allowing the extract to stand one day with a concentrated solution of potassium bichromate. Tannic acid is seldom used as a protein precipitant in this country. The widespread

occurrence of tannins in plant tissue, however, makes it imperative that their effect be removed before estimation of amino nitrogen. This is particularly important when tissues containing but a small amount of amino nitrogen are analyzed.

Limits of error in amino nitrogen determination in different tissues

In order to gain some information concerning the limits of error in amino nitrogen that may normally occur in tissues frequently analyzed, a number of such tissues were extracted with water and 80 per cent. alcohol and the amino content estimated before and after distillation with calcium oxide. The total soluble, non-protein nitrogen was also determined. The results appear in table III.

Without exception distillation with calcium oxide reduced the amount of "amino" nitrogen ranging from 6.1 to 84.9 per cent. of the values deter-

TABLE III

Total soluble, non-protein nitrogen, and amino nitrogen in various plant extracts before and after distillation with calcium oxide. Results expressed as mg. per 100 gm. of fresh tissue

Tissue	Method of extraction	Soluble non-protein	Amino nitrogen in plant extracts			
			Before distillation	After distillation	Decrease	Percentage decrease
		mg.	*mg.*	*mg.*	*mg.*	%
Cabbage plants	80% alcohol	23.8	14.3	10.8	3.5	24.5
	Water	51.5	23.6	19.3	4.3	18.2
Clover tops	80% alcohol	75.6	32.8	29.2	3.6	11.0
	Water	133.3	47.9	44.6	3.3	6.9
Clover roots	80% alcohol	117.2	48.7	41.7	7.0	14.4
	Water	170.2	60.7	56.2	4.5	7.4
Soy bean plants	80% alcohol	105.7	43.0	39.0	4.0	9.3
	Water	163.0	55.4	52.0	3.4	6.1
Sunflower plants	80% alcohol	26.9	6.1	4.6	1.5	24.6
	Water	34.7	10.9	9.5	1.4	12.8
Begonia petioles	80% alcohol	24.3	9.3	1.4	7.9	84.9
	Water	42.9	11.3	3.9	7.4	65.5
Begonia leaves	80% alcohol	15.4	8.6	3.9	4.7	54.7
	Water	32.2	10.6	5.5	5.1	48.1
Rhubarb petioles	80% alcohol	65.0	17.4	3.2	14.2	81.6
	Water	77.8	19.1	6.7	12.4	64.9
Rhubarb leaves	80% alcohol	59.0	24.6	10.6	14.0	56.9
	Water	99.3	30.3	18.1	12.2	40.3

mined before distillation of the extracts with calcium oxide. Distinct tissue differences are evident which must depend upon the amount of non-amino substances free to react in the determination. In most cases good agreement is evident between the decrease in amino value of both water and alcoholic extracts of the same tissue, in spite of the fact that greater amounts of amino nitrogen were invariably removed by the water. Since the size of the aliquot distilled represented a much greater proportion of the total ex-

TABLE IV

AMMONIA AND AMINO NITROGEN IN EXTRACTS OF NORMAL AND PHOSPHORUS DEFICIENT TOMATO PLANTS. RESULTS EXPRESSED AS MG. PER 100 GM. OF TISSUE, FRESH WEIGHT BASIS

TISSUES	GROWTH CONDITIONS	AMMONIA IN EXTRACTS	AMINO NITROGEN IN EXTRACTS			
			BEFORE DISTILLATION WITH CaO	AFTER DISTILLATION WITH CaO	DECREASE	PERCENTAGE DECREASE
		mg.	*mg.*	*mg.*	*mg.*	%
Upper leaves	Normal	11.7	20.3	14.1	6.2	30.5
	Phosphorus deficiency	22.6	25.9	16.1	9.8	37.8
Lower leaves	Normal	13.5	20.8	14.2	6.6	31.7
	Phosphorus deficiency	19.3	27.2	19.2	8.0	29.4
Upper stems	Normal	19.4	25.4	18.0	7.4	29.1
	Phosphorus deficiency	29.3	31.4	21.2	10.2	32.5
Lower stems	Normal	29.9	40.9	30.1	10.8	26.4
	Phosphorus deficiency	83.3	65.2	37.2	28.0	42.9
Roots	Normal	13.5	20.7	13.7	7.0	33.8
	Phosphorus deficiency	26.4	26.3	17.1	9.2	35.0

tract in the case of the alcoholic extracts than with the water extracts, the possibility of the decrease being due to occlusion or destruction of the amino acids through errors in the determination seems precluded. The percentage decrease of amino nitrogen was always greater in the case of the alcoholic extracts, owing to the smaller original amino content.

The analyses reported in tables I, II, and III were carried out with freshly extracted tissues. Accordingly it seemed desirable to investigate the behavior of plant extracts which had been subjected to typical laboratory con-

ditions. Tomato plants were grown in a complete nutrient solution and in a solution deficient in phosphorus, during the spring of 1932, the continuous renewal type of water culture being used. The plants were sampled for analysis in June, using the alcohol preservation method which has been described. Each series of plants was divided into upper and lower stems, upper and lower leaves, and roots. The primary branches were included with the stems, secondary branches and petioles with the leaves. The samples were extracted eight times by the decantation method during October, 1932. The extracts were made to definite volumes, sealed, and allowed to stand six months. At the end of this period suitable aliquots were withdrawn and analyzed for amino nitrogen before and after distillation with calcium oxide. The amount of ammonia present in the extracts was determined by the SESSIONS and SHIVE aeration method (**8**). The results are shown in table IV.

A considerable difference exists between normal and phosphorus deficient plants in ammonia content. Indeed the ammonia content of the deficient plants is greater than their amino nitrogen content, which in turn is greater than the amino content of the normal plants. Such a large amount of ammonia would be expected to give erroneous amino values and the data indicate that this is the case, the amount of error ranging from 26.4 to 42.9 per cent. of the direct determination. The amino values after distillation are not greatly different in the normal and in the phosphorus deficient plants, whereas the direct determination exhibited much wider differences, particularly in the lower stems. It is obvious that determining the amino nitrogen in the presence of appreciable amounts of ammonia results in an overlapping of the nitrogen fractions. As a result the residual or "other" nitrogen is too low. At the present time investigators assign considerable importance to this fraction in the interpretation of metabolism studies. In the stems and roots the concentration of ammonia is sufficient to account for the decrease in "amino" nitrogen after distillation. In the leaves, however, other substances must have been present which added to the ammonia error.

Effect of alcoholic storage on amino nitrogen

Recently WEBSTER (**14, 15**) has reported that stored alcoholic extracts of plant tissue usually increase in ammonia and decrease in amino nitrogen. In WEBSTER'S work the free ammonia was apparently not removed from the extract before making the amino determination. Since the ammonia content of the extracts increased, the direct determination of amino nitrogen should show an increase as well unless the ammonia arises through deamination of the alpha-amino groups. If the ammonia does increase in this manner, then extracts should show even greater decreases after the removal of this ammonia than when the determinations are made without such treat-

ment. Furthermore, the question naturally arises whether or not the observed decrease in amino nitrogen may be due to transformations of the non-amino reacting substances other than ammonia.

Preliminary experiments designed to throw some light on these questions have been carried out with alcoholic extracts of tomato leaves and stems stored in light and darkness. The results of periodic analyses clearly confirm the conclusions of WEBSTER. In one instance with extracts of tomato stems the amino content decreased 35.8 per cent. in the dark and 47.3 per cent. in the light during a storage period of five months. The ammonia content, estimated before making the amino determinations, steadily increased. The rate of increase in ammonia and decrease of amino nitrogen was most rapid during the first four weeks of storage. The loss of amino nitrogen was far greater than could be accounted for by the increase in ammonia. This decrease must represent an actual change in the amino groups so that they are not free to react with nitrous acid. The mechanism of the change is not clear. It is probable that considerable variation might exist in other tissue extracts. Also the rates might be somewhat different during storage in alcohol before complete extraction. However, the common practice of preserving large numbers of samples which cannot be analyzed for long periods of time must be questioned.

Comparison of water and 80 per cent. alcohol as extractives of soluble nitrogen

The data contained in tables I, II, and III permit a direct comparison to be made between water and 80 per cent. alcohol as extractives of soluble nitrogen and amino nitrogen. Without exception extraction with water removed larger amounts of nitrogen than did alcohol. In fact, the magnitude of the differences raises some question as to the value of 80 per cent. alcohol as an extractive of the nitrogenous substances. In some instances 50 per cent. alcohol has proved to be about as effective as water for extraction of the soluble nitrogen. In nitrogen partition experiments with potato tubers it was established that 97 per cent. of the non-protein nitrogen removed with water could be extracted from similar samples with 50 per cent. alcohol by volume. In such cases the tissue is best preserved in 80 per cent. alcohol, the subsequent extractions being made with 50 per cent. alcohol. It would seem that this method (**1**) is deserving of further study and use when large numbers of samples must be handled in a limited period of time.

The writer has frequently found that the excess of total soluble, non-protein nitrogen removed with water over that removed with 80 per cent. alcohol is reflected in higher concentrations of all the soluble fractions, with the exception of ammonia or other volatile bases measured as ammonia. This difference is usually most pronounced in the basic fraction. The in-

crease is not due to incomplete removal of the soluble protein from the water extracts, as preliminary experiments with other protein precipitants, acetic acid, trichloracetic, etc., gave entirely similar results. The possibility of proteolytic enzyme activity was restricted to a minimum since in this work duplicate samples of but a single tissue were taken for extraction at a time, thus permitting prompt treatment of the extracts. It is realized that not all tissues in their fresh state lend themselves to extraction with water. However, it seems desirable whenever possible in nitrogen partition studies to employ fresh tissue and water extraction.

Discussion

In this paper evidence has been presented indicating that the conventional methods for the determination of amino nitrogen in plant extracts may give erroneous results. It has been shown that the preliminary distillation with calcium oxide as outlined made possible the determination of amino nitrogen values which more closely approached the true values. The importance of this treatment depends upon the tissue involved. In a comparatively wide range of plant tissues there was a decrease in amino nitrogen when distilled with calcium oxide, but these data must be regarded as relative rather than absolute. While the composition of any species is doubtless fairly well defined, wide fluctuations in the proportions of the various fractions may occur through differences in physiological age, mineral and organic nutrition, etc. That the amount of amino nitrogen as well as of the non-amino substances concerned will be influenced is certain. Aside from the errors involved in the actual amino determination, it is evident that the preservation and extraction of the tissue may greatly influence the results. The necessity of a careful preliminary study of the properties of a tissue before undertaking extensive analytical work must be emphasized.

Summary and conclusions

1. Treatment of plant extracts with neutral lead acetate, decolorizing carbon, and solid calcium oxide invariably resulted in a decrease of gas measured as amino nitrogen by the Van Slyke method. Evidence is presented supporting the validity of the method of low-temperature distillation with solid calcium oxide under reduced pressure which resulted in maximum decrease in all cases.

2. Acid derivatives of the phenols, as the tannins, were found to react with nitrous acid in the amino determination producing gases measured as nitrogen. This error is eliminated by distillation with calcium oxide. Limits of error caused by the presence of ammonia are discussed.

3. Reductions in gas measured as amino nitrogen ranging from 6.1 to 84.9 per cent. were found in twelve plant tissues extracted with water and

80 per cent. alcohol after distillation with calcium oxide. Five plant fractions of normal and phosphorus-deficient tomato plants showed similar decreases of 26.4 to 42.9 per cent. after ten months' storage in alcohol.

4. Alcoholic storage of tomato plant extracts was found to result in marked increases in ammonia nitrogen and decreases in alpha-amino nitrogen.

5. With nine plant tissues 80 per cent. alcohol extracted an average of only 63.7 per cent. as much soluble non-protein nitrogen and 66.9 per cent. as much alpha-amino nitrogen as was removed with distilled water at a temperature of 25° C.

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GROWTH, ORGANIC NITROGEN FRACTIONS, AND BUFFER CAPACITY IN RELATION TO HARDINESS OF PLANTS[1]

S. T. DEXTER[2]

Introduction

Since the evidence in the literature has become somewhat confused upon several points relating to winter hardiness in plants, it seemed desirable to investigate some of the points of controversy according to a newer technique and with the benefit of more specific information that has recently become available. The newer emphasis, that hardiness does not exist as such in a hardy variety, but that it must be developed, has been the result of the work of a number of investigators. Within the past year or two it has been shown that winter wheat plants of the most hardy varieties harden only poorly when placed at a low temperature in the dark (**15, 3**). The whole matter of increase in resistance to cold has been connected with opportunity for photosynthesis, storage of organic foods, low respiration, and slight vegetative growth (**3, 7**).

Experimentation

In the first experiment it seemed desirable to know whether winter wheat plants added materially to their dry-matter content if stored in a cold-room with artificial illumination. Minhardi plants were grown to an age of three weeks in the greenhouse, after which a part of the crop was moved to a room at 2° C.

In all experiments reported in this paper the plants were grown in quartz sand cultures with nutrient solutions. Six pots with 25 plants each were harvested prior to cold-room treatment, six were stored in the dark at 2° C., and six were stored in continuous light at 2° C. After two weeks the plants in the cold-room were harvested. The hardiness of the plants was determined before and after storage by the freezing-exosmosis method of DEXTER *et al.* (**6**). Table I shows the results of this experiment. Since the pots were very uniform, only totals are given.

From the table it would appear that the plants made marked growth, as seen by the more than doubling of the weight of the dry matter contained in them. It was not possible, however, to distinguish the two sets visually after removal from the cold-room. Increase in the length of the leaves was hardly more than the experimental error. The plants that were stored in the light in the cold-room were materially lower in total nitrogen per

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TABLE I

GROWTH OF WINTER WHEAT (MINHARDI) AT 2° C. SHOWING CHANGES IN HARDINESS WHEN ILLUMINATED AND WHEN STORED IN DARKNESS

SAMPLE	TOPS				ROOTS		TOTAL DRY WT.	FREEZING INJURY. SPECIFIC CONDUCTIVITY $\times 10^3$, 25° C.
	DRY MATTER	GREEN WT.	DRY WT.	TOTAL NITROGEN	GREEN WT.	DRY WT. LESS ASH		
	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
At the start	14.0	51.7	7.2	0.218	42.4	4.8	12.0	99.1
14 days 2° C. dark	14.1	55.3	7.8	0.267	40.7	4.7	12.5	98.5
14 days 2° C. light	22.9	74.3	17.1	0.444	54.8	9.1	26.2	40.0

gram dry matter than the other sets, although the total nitrogen per plant was much greater in them. It is evident that the plants which received illumination carried on active photosynthesis and took up nitrogen from the soil at the temperature of 2° C. These plants hardened materially, whereas the hardening in the plants stored in the dark was hardly perceptible. Analysis showed that the content of reducing sugars in the plants stored in the dark had dropped to a mere trace during the period in the cold-room.

In the next experiment, winter wheat plants were grown for two weeks in the greenhouse with a full nutrient solution. At the end of that time the nitrogen was washed from half the pots, and the plants were allowed to grow for two weeks without nitrogen in the nutrient medium. Calcium chloride was substituted for calcium nitrate. At the end of two weeks without nitrogen, extracts from the minus-nitrogen plants gave no test for nitrates with diphenylamine and the plants were presumed to be virtually free from inorganic nitrogen. They showed every evidence of nitrogen deficiency.

By means of this experiment it was hoped that several points might be clarified: (1) Does the commonly reported increase in soluble organic nitrogen result from uptake from the soil, or is it due to breakdown of proteins? (2) Does the winter wheat plant require nitrogen in the soil during hardening? (3) Will minus-nitrogen winter wheat plants harden at a low temperature in the dark?

The organic nitrogen fractions in the plants were determined in the following way. Fresh samples (20 plants) were treated with ether and

ground to a smooth paste with quartz sand. The sample was made to a definite volume with tenth normal potassium sulphate, centrifuged, and the residue washed and centrifuged three times. The resulting suspension was coagulated with heat, after adding a few drops of acetic acid. The coagulated material was removed by filtration. The three fractions were determined separately, *i.e.*, the soluble or non-coagulable fraction, the coagulable fraction, and the fraction thrown out by the centrifuge. It was not found possible to obtain checks on duplicates for the latter two determinations, although the "soluble" fraction gave good checks. The "soluble" nitrogen is reported, then, as the percentage of the total nitrogen in the plants. On this basis the duplicate samples checked well. Table II gives the results of this experiment. The experiment was repeated, with the additional analyses for amino nitrogen (Van Slyke), and for carbohydrates. Table II includes these figures. According to a similar idea, samples of cabbage leaves were prepared from plus-nitrogen plants, deficient in starch and actively growing, and from minus-nitrogen plants high in starch. Samples to be compared in any regard consisted of half-leaves, split at the midrib. One set was analyzed as it came from the greenhouse, the other after storage in a moist chamber at 2° C. in the dark for 7 days.

Table II appears to answer the first question as to the increase in soluble organic nitrogen in plants stored at a low temperature. Several workers have noted this increase and have attributed the increase in hardiness partly to this cause (**10, 6**). In a recent paper NEWTON *et al.* (**12**) concludes that the increase in soluble nitrogen, especially in alpha amino form, is due to the occasional freezing of the plants during the early winter season. The plants in this experiment were not frozen, however. That the increase in soluble nitrogen comes from the breakdown of proteins seems probable from the figures presented in the table. Plants which gave no tests for nitrate nitrogen increased as much in soluble nitrogen as those liberally supplied with the element in nutrient solutions, and in which liberal amounts were present, according to the diphenylamine test.

Table II presents the data relating to hardening of these plants at 2° C. both in light and in darkness. Since it was anticipated that the samples might differ greatly in total soluble extractable electrolytes, samples were killed by heating and extracted to give the total salts in the same volume of water as was used for the freezing-exosmosis test (**4**). The samples of winter wheat crowns were frozen at −8° C. for two hours; the cabbage at −6° C. for two hours. The table shows that winter wheat plants high in carbohydrates, due to nitrogen starvation, hardened well in the cold room. Those in the light hardened more than those in the dark, but in either case mineral nitrogen did not seem to be necessary for the hardening process. High-carbohydrate plants of either wheat or cabbage hardened well in the

TABLE II

ORGANIC NITROGEN FRACTIONS IN PLUS- AND MINUS-NITROGEN PLANTS BEFORE AND AFTER EXPOSURE TO LOW TEMPERATURE (2° C.)

SAMPLE*	DAYS AT 2° C.	ILLUMINATION	PERCENTAGE N IN SOLUBLE FORM	PERCENTAGE N IN AMINO FORM	CARBOHYDRATES AS PERCENTAGE DRY MATTER			SPECIFIC CONDUCTIVITY ($\times 10^5$, 25° C.) AFTER	
					REDUCING	SUCROSE	STARCH	FREEZING INJURY	HEATING (TOTAL SALT)
			%	%	%	%	%		
Winter wheat									
Plus-N	0	.....	25.3	.....	.....	.....	.....	75.7	116.9
	5	Light	18.3	.....	.....	.....	.....	48.5	116.5
		Dark	28.6	.....	.....	.....	.....	72.5	116.2
	10	Light	26.2	.....	.....	.....	.....	31.1	97.2
		Dark	31.9	.....	.....	.....	.....	69.1	115.0
Minus-N	0	.....	21.1	.....	.....	.....	.....	65.1	103.5
	5	Light	22.1	.....	.....	.....	.....	33.6	85.7
		Dark	26.9	.....	.....	.....	.....	49.7	98.7
	10	Light	24.3	.....	.....	.....	.....	25.6	83.8
		Dark	30.7	.....	.....	.....	.....	39.6	92.8
Repeated									
Plus-N	0	.....	20.0	9.94	.....	.....	.....	.....	.....
	10	Light	29.1	17.9	.....	.....	.....	.....	.....
		Dark	33.1	14.2	.....	.....	.....	.....	.....
Minus-N	0	.....	19.7	4.98	1.85	4.8	5.42	52.8	88.2
	10	Light	22.5	10.60	2.91	20.55	12.26	22.5	75.3
		Dark	30.0	16.95	3.88	11.12	3.41	29.9	85.8
Cabbage				.....	Total sugars				
Minus-N	0	.....	8.80		22.2			67.4	181
	7	Dark	17.46		29.0			37.4	160
Plus-N	0	.....	13.96		No test for starch qualitatively			126.5	227
	7	Dark	19.48					133.6	223

* Part of the winter wheat samples were stored at 20° C. with continuous illumination, part in the dark. All cabbage samples were stored in the dark. Conductivity values for exosmosis from wheat crowns and from cabbage leaves following freezing injury and following heating are included.

dark; low carbohydrate plants little or not at all. If illuminated, plus-nitrogen plants hardened well.

To summarize the results of this experiment it may be stated that: (1) Proteins are split in the plants stored at this low temperature entirely regardless of the hardening process. In every case the plants which hardened least increased most in soluble nitrogen. (2) Cabbage and winter wheat plants do not require nitrogen from outside the plant to carry on the hardening process. (3) Minus-nitrogen plants, high in starch, hardened well at 2° C. without illumination, in sharp contrast with those low in carbohydrates.

Certain elements were not included in the nutrient solutions in the sand cultures. The salts used were calcium nitrate, potassium acid phosphate, and magnesium sulphate, with traces of iron as phosphate. Tests for chlorides in the extracts from the crowns showed mere traces. Seemingly chlorides are not necessary in the hardening process but did not prevent it, since calcium chloride was used in the minus-nitrogen series. The apparent loss of minerals from the samples of winter wheat which harden has been described more fully in another paper (**4**).

It seemed desirable to investigate the hardening capacity of other minus-nitrogen plants. Winter rye, winter barley, and winter oats were grown as previously described. As in the case of winter wheat, minus-nitrogen plants of rye, barley, and oats were found to harden in the dark at 2° C., whereas plus-nitrogen plants hardened very slightly. To a few pots of plus-nitrogen plants of these four species, a 2 per cent. sucrose solution was added, and the plants set at 2° C. in the dark. With all four cereals hardening was somewhat better than the corresponding plants which did not receive sugar, but not so complete as the minus-nitrogen plants in the dark (**2**). According to a similar idea, the freshly severed stems of cabbage plants were placed in water, in 2.5 and 10 per cent. sucrose solutions, and kept in the greenhouse for 40 hours. The samples were then analyzed for sugars, for freezing injury at – 6° C., and for ice formed, by the calorimetric method. The results were perfectly regular and orderly. More water was left unfrozen in the plants placed in 10 per cent. sugar than in 2.5 per cent., which in turn showed more unfrozen water than the plants kept in water alone. Average water unfrozen in four samples of each was 9.95, 8.70, and 6.94 gm. in the 10 per cent. sucrose, the 2.5 per cent. sucrose, and the water only, respectively. Injuries, according to specific conductivities taken, were 44.5, 59.5, and 75.1 for the samples as just given. The amount of total sugars in the plants kept in 10 per cent. sugar was more than double that in the others. Peculiarly enough the reducing sugars were about twice as great in amount as in the normal plants, although sucrose only was supplied. Evidently the plant has a capacity to hydrolyze rapidly

sugars furnished in this way. The marked protection against freezing injury might well be explained by the decrease in the ice formed. The increase in hardiness, however, was far less than can readily be accomplished by simple exposure to low temperatures for a week, during which time the increase in sugar is far less than was found in these cases. It seems improbable that increase in hardiness can usually be explained in such simple terms.

In another experiment, the buffer capacity of samples of cabbage leaves before and after hardening was investigated. Half-leaf samples were again prepared from plants high and low in carbohydrates respectively. One set of half-leaves was ground with a pinch of quartz sand before exposure to cold; the other half-leaves were put to harden in the cold room for seven days. They were stored in a moist chamber in the dark. After grinding to a smooth paste, the samples were made to a definite volume, and the hydrogen ion concentration determined with a hydrogen electrode. Definite volumes of tenth normal hydrochloric acid or sodium hydroxide were added with a pipette to half the sample, and the hydrogen ion concentration again determined, until five additions of acid or base had been made. A total of eight samples of each type of sample was run, on two occasions. The voluminous data obtained will not be presented in full. In seven out of eight samples of cabbage leaves which actually hardened at 2° C., the ground leaves were found to be slightly more alkaline after exposure to cold than before; in six out of eight samples of low carbohydrate leaves, which did not actually harden at 2° C., the leaves were found to be slightly more alkaline after exposure to cold than before. Thus whether the plants hardened or failed to harden, this response appears to be more or less identical. The changes were practically the same in each case and amounted to about 0.2 pH unit. In all cases, however, the plants that were high in carbohydrates were distinctly more acid than those low in carbohydrates. Those high in nitrogen were found to be better buffers, as well, either before or after exposure to cold. Exposure to cold did not appear to change the buffer capacity in either case.

The increase in hardiness has been attributed to an increase in soluble nitrogen and an increase in buffer capacity. An increase in soluble nitrogen unquestionably occurs on exposure to low temperatures, but it occurs to a greater degree in plants that have not hardened than in those which have. NEWTON (**11**) failed to find a correlation between buffer capacity and hardiness in winter wheat. HARVEY (**8**), who reported a definite increase in buffer capacity in cabbage which was hardened, used the expressed juice from the plants. This expressed juice, after hardening, is usually higher in total solids than before hardening, and might well show more buffer capacity since it contains more dry matter. In the technique

used in this experiment, however, virtually the same amount of dry matter was used in each case. No change in buffer capacity was found in a given number of cells or a given amount of material. The increase in soluble nitrogen in high carbohydrate plants during exposure to cold is not particularly great, since the quantities present are small. In the samples analyzed, less than 0.3 mg. of soluble nitrogen was present per gram of fresh leaves. Even though this amount almost doubles during exposure to cold, it must still be a very small factor in the buffering of the plant juice. The amount of soluble nitrogen present in the vegetatively active cabbage plants was about four times this amount.

In view of the findings of these previous experiments, it seemed desirable to reinvestigate the matter of the hardening of plants in an alternating

TABLE III

CHANGE IN HARDINESS, SOLUBLE NITROGEN, AND SUGARS IN HALF-LEAF SAMPLES OF CABBAGE STORED FOR SEVEN DAYS IN THE DARK AT CONSTANT LOW, ALTERNATING HIGH-LOW, AND INTERMEDIATE TEMPERATURES

Sample	Freezing injury. Specific conductivity $\times 10^{-5}$			Soluble N		Sugars					
						Reducing			Sucrose		
	2°	2°–20°	15°	2°	2°–20°	2° C.	2° C.–20° C.	15° C.	2° C.	2° C.–20° C.	15° C.
	°C.	°C.	°C.	mg.	mg.	gm.	gm.	gm.	gm.	gm.	gm.
1	236	595	621	.....	.....	.....	.....	.....	.....	.....	.....
2	172	462	508	.....	.....	.....	.....	.....	.....	.....	.....
3	140	281	264	.....	.....	.....	.....	.....	.....	.....	.....
4	271	552	604	.....	.....	.....	.....	.....	.....	.....	.....
5	419	419	286	.....	.....	.....	.....	.....	.....	.....	.....
Average	247	462	457	15.1	41.1	0.613	0.543	0.549	0.272	0.140	0.099

temperature. HARVEY (**9**), TYSDAL (**16**), and others in unpublished work, have observed increased hardening in plants that were subjected to alternating temperatures. Frequently these plants were given continuous illumination, whether at a constant or an alternating temperature. DEXTER (**3**) and TYSDAL (**16**) have used various lengths of day and night during hardening. DEXTER has presented data which seem to indicate that the increased hardening under such conditions is due to increased photosynthesis and greater net storage of organic foods. In the experiment now reported, half-leaf samples were stored at 2° C. continuously. The other halves were alternated between 2° and 20° C. at approximately 12-hour intervals. A third sample, as nearly like the duplicate half-leaf samples

as possible, was stored at a constant temperature of 15° C. It was estimated that the respiration of this sample would be approximately that of the sample alternated between 2° and 20° C. The samples were contained in moist chambers, in the dark, and no wilting was evident at the end of the experiment although those at the higher temperatures were somewhat yellowed. Five samples were stored under each temperature condition for examination of hardiness. Samples were also prepared for examination of change in soluble nitrogen and for sugar analysis. Table III presents the data from this experiment.

From the data given in table III, it can be seen that the samples stored at 2° C. hardened a great deal more than duplicate half-leaf samples alternated from 2° to 20° C. Each set was in the dark. The samples alternated appear to be almost precisely as hardy as the samples kept constantly at 15° C. These were not duplicate half-leaf samples but were as nearly identical as possible. The soluble nitrogen in the leaves kept at 2° C. continuously is less than half that of the alternated leaves. The increase in soluble nitrogen in the alternated leaves evidently did not give them greater hardiness. The sugars, and especially sucrose, were higher in the sample kept continuously at 2° C. than in either of the other two samples. The other two samples agree perhaps as well as could be expected, since they were not duplicate half-leaves. These data would seem to support the idea previously put forth (3) that if greater hardening is found under conditions of alternating temperature, above the freezing point, it is due to the greater photosynthesis under those conditions. Alternating temperatures, with the plants in the dark, or in the light without carbon dioxide, do not seem to stimulate the hardening reaction.

Summary and conclusions

1. A reexamination of some of the theories of winter hardiness of plants has been made. It should be recognized in any work dealing with the hardening reaction, that plants which are usually thought of as hardy need not necessarily harden if placed at a low temperature. This reaction seems to be largely dependent upon the organic nutrition of the plant, if opportunity for photosynthesis is denied at the low temperature.

2. Plants of a hardy variety of winter wheat were grown with excess and with minimum nitrogen supplies in the nutrient medium. When placed at 2° C. without illumination, it was found that those high in nitrogen did not harden, although there was a considerable increase in soluble organic nitrogen in the plants during the period at the low temperature. The minus-nitrogen, high-carbohydrate plants hardened well in the dark with an increase in soluble organic nitrogen. Sugars increased in the high-carbohydrate plants and decreased in the plus-nitrogen plants during

such storage. Although the behavior in regard to organic nitrogen was almost indistinguishable, the difference in hardening was marked. With both sets of plants in the light at a low temperature, hardening was efficiently carried out, and the increases in soluble organic nitrogen were less than with corresponding plants in the dark, where hardening was in all cases less. Thus increase in soluble organic nitrogen is no indication of increase in hardiness.

3. Minus-nitrogen, high-carbohydrate plants of winter rye, winter barley, winter oats, and cabbage were found to harden at 2° C. in the dark, while corresponding plants low in carbohydrates due to surplus nitrogen in the nutrient medium did not harden under those conditions.

4. Cabbage leaves, low in carbohydrates and high in nitrogen, although they hardened but little at a low temperature were found to be more alkaline and better buffers, both before and after exposure to cold, than leaves high in carbohydrates and low in nitrogen. While the samples of both sets were generally slightly more alkaline after exposure to cold for a week, they were not distinguishably better in buffering capacity than before exposure to cold.

5. Constant low temperature in the dark was more effective in causing hardening in cabbage leaves than alternating temperatures in the dark. Higher sugar content was found in the samples kept constantly at 2° than in samples alternated between 2° and 20° or stored at a constant temperature of 15° C. Soluble nitrogen was higher in the alternated leaves than in those held at 2° C.

6. There appears to be a series of reactions which proceed in a plant at a low temperature entirely regardless of increase in hardiness. There is an increase in soluble organic nitrogen. There is a decrease in respiratory rate which is not correlated with increase or decrease in sugars or enzyme activity (**5, 1**). There is, usually at least, an increase in sugars, which may or may not be accompanied by decidedly increased hardiness. A continued and critical examination of the theories of winter hardiness is needed.

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RELATIONSHIP BETWEEN NITROGEN FERTILIZATION AND CHLOROPHYLL CONTENT IN PINEAPPLE PLANTS[1]

R. K. TAM AND O. C. MAGISTAD

(WITH TWO FIGURES)

Introduction

Since nitrogen is an essential part of the chlorophyll molecule, any appreciable lack of it in the mineral nutrient supply of the plant should result in a suppression of chlorophyll formation. The extent of this suppression would, of course, be modified if there were a deficiency in other growth factors such as light, iron, magnesium, carbon dioxide, and water.

It is the purpose of this paper to show the relationship existing in leaves of pineapple plants between the total chlorophyll ($\alpha + \beta$) concentration and various amounts of nitrogen applied as fertilizer in a series of field experiments.

According to SCHERTZ (**8**), VILLE in 1889 was probably the first investigator to note a definite relationship between nitrogen supply and chlorophyll formation. The color of the plants observed varied according to the amount of nitrogen applied. Similar observations were made by PINCKNEY (**5**) in the case of sorghum fertilized with sodium nitrate.

SIDERIS (unpublished data) has shown that leaves of pineapple plants receiving very large quantities of nitrogen had an increased content of amino acid and chlorophyll.

SCHERTZ (**6**), working with *Coleus blumei* (var. Golden Bedder), found that the mottling of leaves was due to a lack of nitrogen and could be prevented by the addition of sodium nitrate. The addition of iron, magnesium, calcium, or phosphate failed to prevent mottling and subsequent shedding of the leaves by the plant. On the other hand, BRIGGS, JENSEN, and MCLANE (**1**) were unable to establish a relationship between the percentage of leaves mottled and the total nitrogen content in the soil in both the orange and lemon groves studied. In a later investigation SCHERTZ (**8**) concluded that the amount of nitrogen supplied correlated with the amount of chloroplast pigments present in the fresh green leaves of cotton and potato plants.

ULVIN (**10**) recently found that more chlorophyll was produced by plants of sugar cane receiving nitrogen as nitrate than by those receiving nitrogen as ammonia at each of three soil temperatures. The application of 1 to 15 p.p.m. of iron caused a marked increase in chlorophyll formation.

[1] Published with the approval of the Director as Technical Paper no. 77 of the Pineapple Experiment Station, University of Hawaii.

GUTHRIE (3) showed that the availability of nitrates affected the chlorophyll content of plants differently during winter and spring. The lack of nitrates in winter did not induce any chlorophyll loss, but in spring a great reduction occurred when there was an insufficient supply of nitrates.

Experimentation

MATERIAL

Total chlorophyll ($\alpha + \beta$) determinations were made on pineapple leaves from four field experiments. Table I gives the location, number of plants per acre, and fertilization rate in pounds of nitrogen per 1000 plants for each of the treatments in the four experiments. The amount of nitrogen indicated was applied during the growing period of approximately 20 months.

TABLE I

DESCRIPTION OF FIELD EXPERIMENTS FROM WHICH LEAF MATERIAL WAS OBTAINED

EXPERIMENT NO.	LOCATION	PLANTS PER ACRE	FERTILIZATION RATE IN LB. N PER 1000 PLANTS
I	Field 6, Kunia	15,600	0, 6.4, 12.8, 19.2, 25.6, 38.4
II	Field 4719, Opaeula	12,300	0, 8.1, 16.2, 24.3, 32.4, 48.6
III	Field 15a, Wahiawa Substation	9,680	0, 10, 20, 30, 40, 60
IV	Field 45, Maui	17,424	0, 5.7, 25.8, 37.2, 42.9, 48.6

Experiments I, II, and III were each divided into two parts. Part A received its nitrogen in two applications, at the time of planting and at the age of 12 months. Part B received its nitrogen in four applications, one-fourth at the time of planting and equal amounts at the age of 6, 12, and 18 months. The leaf samples were obtained shortly prior to harvest when the pineapple fruits were still green but nearly full size.

All four experiments were installed in the fall (September and October) of 1932. They were regularly and well supplied with iron as ferrous sulphate spray.

Ammonium sulphate was used as the nitrogen source in all four experiments. Neither potassium nor phosphorus was applied in experiments I, II, and III. All of the plots in experiment IV had been uniformly fertilized with 5.8 lb. P_2O_5 and 2.9 lb. K_2O per 1000 plants.

METHOD OF DETERMINATION

The method used in the extraction and separation of chlorophyll from the other plant pigments was that of WILLSTÄTTER and STOLL (11) as

modified by SCHERTZ (7). It was found after a few trials that the great variation in chlorophyll content of plants between treatments made it necessary to vary the size of the samples taken for chemical analysis. Accordingly, 25 gm. were used in the case of the samples from the low nitrogen plots while 15 and 10 gm. samples were used for the plants fertilized with larger amounts of nitrogen. Care was taken to grind the larger samples for a longer period of time than the smaller samples in order to obtain an equal degree of extraction.

Subsequent to extraction, saponification, and separation, the chlorophyllins were run into a 100-cc. volumetric flask and made to volume with distilled water. Determinations were made by comparison, in a Klett colorimeter, with the artificial color standard developed by GUTHRIE (2).

SAMPLING

Before proceeding with the regular analyses of the samples from the different experiments, it was desired to determine the variations existing in the chlorophyll content of plants within the same plot. A knowledge of the degree of the variations would provide information as to the number of leaves to use for a reliable sample.

Single leaf samples from each of twelve separate plants were obtained from the plot receiving 25.6 lb. nitrogen per 1000 plants of experiment IA. Total chlorophyll determinations were made on these twelve samples, and the means, standard deviations, and coefficients of variability were calculated. These appear in table II.

TABLE II

VARIATION IN CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS WITHIN A SINGLE PLOT

STATISTIC	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
	mg.	*mg.*	*mg.*
Mean	61.36	336.65	13.71
Standard deviation, σ_x	± 5.22	± 31.17	± 1.20
Coefficient variability, C. V.	8.51	9.26	8.75

From a knowledge of the standard deviation of these twelve samples (table II) and the great range in chlorophyll content at various nitrogen levels, it was decided that a sample consisting of one leaf from each of three separate plants, within the same plot, would constitute a sufficiently reliable analysis. Accordingly all subsequent samples were composited from three

leaves. The longest leaves were used and fall in group L–III (**9**). At the time of sampling all plants were approximately 20 months old with fruits nearing maturity.

Determination of leaf area

In order to calculate chlorophyll content per unit area, some means of measuring leaf areas was necessary.

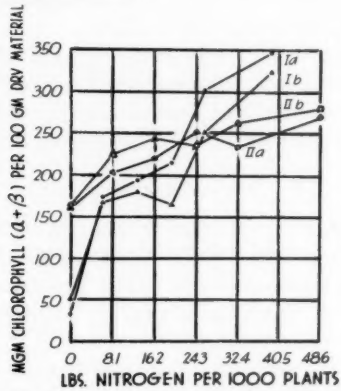

Fig. 1. Chlorophyll ($\alpha+\beta$) content of pineapple leaves with varying amounts of nitrogen applied; dry weight basis.

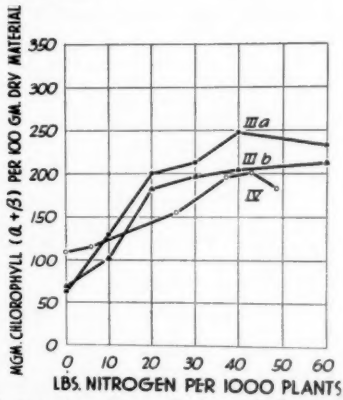

Fig. 2. Chlorophyll ($\alpha+\beta$) content of pineapple leaves with varying amounts of nitrogen applied; dry weight basis.

Krauss (**4**), in studies on the transpiration of pineapple plants, determined the area (upper and lower) of large pineapple leaves by considering them triangles of small base and great altitude, and used the formula: area = length × width.

The width was measured along an imaginary line near the base of the leaf at the junction of white and green tissue. The length was taken from this line to the tip of the leaf.

This formula was used in the present study to determine the area, except that the product of the length times the width was multiplied by 0.5 to obtain the area from one side of the leaf only. The widths were taken 5 cm. from the base of the leaf and the lengths from this point to the tip of the leaf. The formula was checked by a series of planimeter readings of traces of representative leaves. Calculated on the basis of eleven readings, a mean

TABLE III

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT I

	N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
	lb.	*%*	*sq. cm.*	*mg.*	*mg.*	*mg.*
IA	0	80.67	633.33	6.4	33.09	1.02
	6.4	82.61	601.11	30.3	174.23	5.04
	12.8	81.29	530.73	36.5	194.91	6.88
	19.2	81.40	537.44	40.0	215.20	7.44
	25.6	80.69	509.81	58.0	300.44	11.38
	38.4	81.60	477.91	64.0	347.52	13.40
IB	0	81.22	513.27	9.4	50.01	1.84
	6.4	81.35	633.99	31.3	167.77	4.94
	12.8	81.34	458.27	33.5	179.56	7.30
	19.2	81.07	516.84	32.0	168.96	6.20
	25.6	80.41	488.65	49.1	250.16	10.02
	38.4	82.14	488.98	58.0	324.80	11.62

deviation of 3.16 per cent. was obtained between actual planimeter readings and values obtained through the use of this formula.

After discarding the basal 5 cm. of white tissue, the total wet weights of the three leaf samples were recorded. The leaves were then ground in a meat chopper and their total moisture determined. The total chlorophyll determinations were begun immediately after the samples were ground.

RESULTS

The results of the determinations of the chlorophyll content of the samples from the four field experiments are found in tables III, IV, V, and

VI. Chlorophyll concentrations have again been calculated on both the wet and dry weight bases and also on the 100 sq. cm. wet basis.

Figures 1 and 2 show the graphic relationship existing between the amount of nitrogen applied and the chlorophyll concentration of plants from the various plots in the four experiments. Chlorophyll values obtained on the wet weight basis were used in the construction of these curves.

TABLE IV

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT II

N PER 1000 PLANTS		MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
lb.		*%*	*sq. cm.*	*mg.*	*mg.*	*mg.*
IIA	0	83.57	525.78	26.57	161.81	5.06
	8.1	84.71	567.92	31.10	203.39	5.48
	16.2	82.94	550.54	37.50	219.75	6.82
	24.3	82.49	526.55	43.97	251.07	8.36
	32.4	82.50	496.81	41.13	234.85	8.28
	48.6	81.77	515.38	49.04	269.23	9.52
IIB	0	83.15	606.25	27.72	164.38	4.58
	8.1	83.37	543.02	37.50	225.38	6.90
	16.2	83.68	481.59	39.85	244.28	8.28
	24.3	82.59	534.03	41.13	236.09	7.70
	32.4	82.07	534.70	47.22	263.49	8.84
	48.6	80.98	499.65	53.13	279.46	10.64

Discussion

Natural variation in concentration of chlorophyll in leaves from a single plot is appreciable, as shown in table II. On the weight basis, the standard deviation of a determination was $\pm$ 5.22 with a coefficient of variability of 8.51 per cent. This coefficient of variability was the lowest of the three that were obtained on the different bases. Obviously, variations in moisture contents and discrepancies between weights and areas contributed to the higher values obtained on the dry weight basis and on the area basis.

Dividing the standard deviation of $\pm$ 5.22 by $\sqrt{3}$, a value of $\pm$ 3.01 is obtained. This is the standard error of a determination using a composite sample of three leaves, one each from separate plants within a plot. With

TABLE V

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT III

N PER 1000 PLANTS		MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
lb.		%	*sq. cm.*	*mg.*	*mg.*	*mg.*
IIIA	0	83.57	473.37	10.62	64.68	2.24
	10	82.90	473.19	22.14	129.52	4.68
	20	82.38	442.00	35.40	201.07	8.00
	30	82.54	460.52	37.50	214.88	8.14
	40	81.69	545.28	45.55	248.70	8.36
	60	80.54	523.91	45.55	234.13	8.70
IIIB	0	84.05	449.22	11.08	69.47	2.48
	10	82.68	501.37	17.68	102.01	3.52
	20	82.64	476.20	31.90	183.74	6.70
	30	82.56	458.29	34.45	197.40	7.52
	40	81.77	491.36	37.50	205.88	7.64
	60	81.33	494.68	59.85	213.60	8.06

TABLE VI

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT IV

N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
lb.	%	*sq. cm.*	*mg.*	*mg.*	*mg.*
0	81.26	578.66	20.71	110.59	3.58
5.7	81.23	633.49	21.78	116.09	3.44
25.8	81.16	558.79	29.27	155.42	5.24
37.2	81.41	582.48	36.45	196.10	6.26
42.9	81.40	562.17	37.50	201.75	6.68
48.6	81.25	544.82	34.45	183.62	6.32

large differences in chlorophyll content for the various treatments, the sampling error becomes relatively small.

Figures 1 and 2 show graphically the relationship existing between the increase in chlorophyll content and the increase in nitrogen fertilization. Increases were noted in all four experiments. In general, with every increase in the amount of nitrogen applied a corresponding increase in the chlorophyll concentration was noted. There were slight deviations from the rule, however. These exceptions were experiment IB with the 19.2 lb. per 1000 plants application; IIA with the 32.4 lb. application; and IIB with the 24.3 lb. application. A decrease in the chlorophyll content of the plants of the plot fertilized with the highest amount of nitrogen occurs in experiment IIIA, and may possibly be due to a state of excessive nitrogen fertilization.

The chlorophyll concentrations of the plants receiving no nitrogen in the four experiments varied considerably. Experiments I and III showed the lowest chlorophyll concentrations, while samples from experiment II contained the highest amount of chlorophyll of the four experiments. These different levels of chlorophyll concentration would seem to be correlated with the differences in nitrogen availability between the areas in which the experiments were located. However, analyses of soils from these plots for available nitrogen were not made.

The greatest response, from the standpoint of chlorophyll increase, was recorded in experiment IA. The range, from the plot receiving no nitrogen to that receiving 38.4 lb. per 1000 plants, was from 33.09 to 347.52 mg. of chlorophyll per 100 gm. dry material. The increase was more than tenfold. Field observations of the plots in this experiment indicated such a great increase to be possible. The plants in the plots receiving no nitrogen were decidedly chlorotic and underdeveloped, while those receiving the high nitrogen applications were large, dark green in color, and extremely healthy. Fruiting in the former case was also conspicuously retarded. Incidentally, samples from the plot with no nitrogen applied in this experiment gave the lowest chlorophyll concentrations of all four experiments.

Experiment III also showed a good response to nitrogen fertilization. Although the maximum amount of nitrogen applied was greater than in experiment I, the total chlorophyll concentration was not so great.

The least response to nitrogen fertilization was noted in experiments II and IV. The chlorophyll levels in the plots receiving no nitrogen were high in both experiments. In both cases the total chlorophyll concentrations of the plants from the plots receiving the highest amount of nitrogen were less than twice that of the plants from the plots receiving no nitrogen.

The number of applications of nitrogen had little effect on chlorophyll content. Except for experiment II, the chlorophyll determinations of the

plants receiving the nitrogen in two applications (A) showed slightly higher chlorophyll concentrations. This was most probably due to the fact that the plants in these plots received their full quota of nitrogen sooner than the plants receiving it in four applications (B). Applying the nitrogen at an earlier period allowed this difference in nitrogen availability between early and late applications to become a factor in chlorophyll formation up to the time of the analysis. The small response in chlorophyll formation due to nitrogen fertilization in experiment II was probably the cause for this exception.

Summary

Leaf samples from four field experiments, fertilized with varying amounts of nitrogen, were analyzed for their total chlorophyll ($\alpha + \beta$) concentrations. Variations in the total concentrations were noted between different experiments and between plots within the same experiment. With few exceptions, an increase in the amount of nitrogen applied resulted in a corresponding increase in the total chlorophyll concentration. This would indicate that, in pineapple plants, the amount of available nitrogen present determines to a large degree the amount of chlorophyll formed, provided the other requirements for chlorophyll formation, such as light, iron supply, and magnesium, are present in sufficient quantities.

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A MECHANISM FOR CONTROLLED CONTINUOUS FLOW OF NUTRIENT SOLUTIONS[1]

F. P. MEHRLICH

(WITH FIVE FIGURES)

Introduction

The composition of a nutrient medium is constantly changed by the plants growing in it. Constant renewal of the nutrients is therefore imperative if the plant condition is to be correlated with a known composition of medium.

The apparatus for continuously renewing nutrient solutions described in this paper is a modification of SHIVE's device.[2] It is essentially a closed system, difficult for dirt to enter, and has a minimum of free surfaces from which evaporation may occur to concentrate the nutrients. While SHIVE's apparatus has a capacity of 2 or 3 liters and requires daily refilling with consequent disturbing of the siphons, the present device holds at least 38 liters and the reserve nutrient may be renewed repeatedly without upsetting the rate of flow. It is practically self starting, more compact and more rugged than other devices, and is suitable for use in exposed places and in the field.

Since it is wholly separate from the plant contained, whatever container is preferable from other considerations may be used. The container may be moved about for examination or manipulation of the plant without interfering with the rate of nutrient delivery. This apparatus may be used with solution, sand, or soil cultures.

Apparatus

The apparatus is composed of six primary parts: (1) inverted reservoirs; (2) constant-level reservoir; (3) tubulatures connecting the two types of reservoirs; (4) air-tubulature adjustment; (5) capillary siphons leading from the constant level reservoir to the plant containers; and (6) capillary-siphon adjustment. Figure 1 illustrates a single complete unit, showing the relationship of the larger parts. The tubulatures and tubulature adjustments are shown in figure 2.

INVERTED RESERVOIRS.—The inverted reservoirs may be of any size or shape desirable for special needs; five-gallon glass carboys are well suited for most purposes. Provision has been made for the use of two or more such reservoirs per unit of the apparatus described in this paper. Thus an

[1] Published with approval of the Director as Technical Paper no. 78 of the Pineapple Experiment Station, University of Hawaii.

[2] ALLISON, R. V., and SHIVE, J. W. Amer. Jour. Bot. 10: 554–567. 1923.

adequate reserve of nutrient solution may always be maintained without appreciably changing the level of the constant-level reservoir. Ordinarily only one carboy is used at a time. When it is nearly empty the second is filled and put into place. The adjustment to the carboys is set so that the first empties completely before the second one is drawn from. This feature,

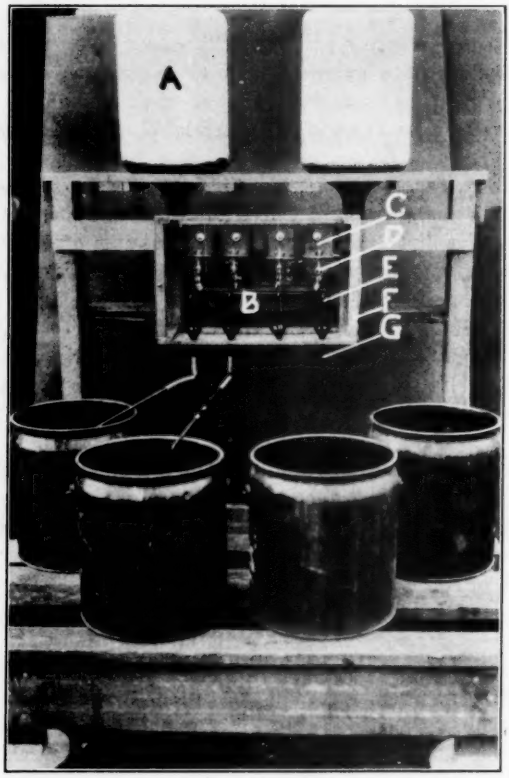

FIG. 1. Single unit of drip apparatus: *A*, inverted reservoir; *B*, constant-level reservoir; *C*, capillary-siphon rough adjustment; *D*, capillary-siphon fine adjustment; *E*, capillary-siphon; *F*, siphon shield; *G*, funnel. The front of the siphon shield and the funnel cover were removed prior to photographing.

however, makes possible the refilling of the two carboys almost simultaneously if special need requires it. Thus a week's supply may be provided at one time. For certain purposes it may seem more desirable to use only one inverted reservoir, or to connect a number of them in trains as described under a separate heading.

The carboys and glass tubulatures are painted first black and then white, to inhibit the growth of algae and to minimize temperature fluctuations.

CONSTANT-LEVEL RESERVOIR.—Tests have shown that the inverted reservoirs (carboys) give out slightly increased quantities of solution as they become progressively empty. Using a small volume constant-level reservoir, such as a glass tumbler, fluctuations in level as great as 2 cm. may occur, upsetting the rate of flow to the plant containers. A large surface reservoir such as that pictured in figures 1 and 2 minimizes this fluctuation. Glass trays or graniteware pans 12 inches by 8½ inches in size are recommended.

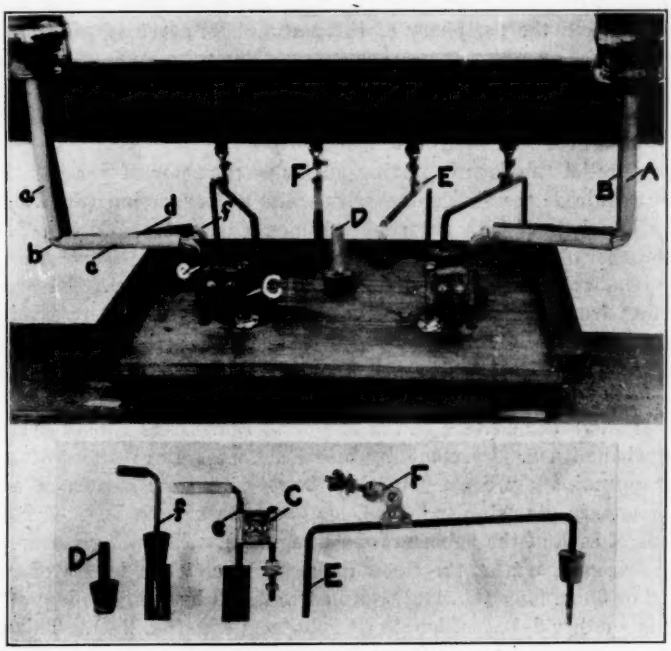

FIG. 2. Constant-level reservoir and fittings: *A*, air channel between inverted reservoir and constant-level reservoir; *B*, solution channel between the two types of reservoirs; *C*, air-tubulature adjustment; *D*, vent tube; *E*, capillary-siphon; *F*, capillary-siphon fine adjustment; *a–e*, various sections of air channel; *f*, final section of solution channel.

Their use reduces the maximum fluctuation to 2 mm. A cover of 1½-inch pine wood or ⅜-inch ply wood has been used successfully. A number of holes of suitable diameter to admit a no. 3 rubber stopper are cut in it to accommodate as many capillary siphons as are desired (fig. 2). At each end of the cover three holes are cut, one to accommodate a no. 3 rubber stopper

fitted with the tubulature for carrying solution from the inverted reservoir (fig. 2 *f*). The other two holes are for the adjustment of the air inlet to the inverted reservoir (fig. 2 *e*, *C*). Still another opening is cut in a central position to admit a no. 3 stopper carrying a small glass tube loosely plugged with glass wool (fig. 2 *D*). This tube may be withdrawn for cleaning the apparatus or to make possible a measurement of solution level in the reservoir. It also maintains the system at atmospheric pressure.

After all holes are made, the cover is waterproofed with hot paraffin. The tubulatures are adjusted to the proper depth and the cover is bolted in position to the shelf supporting the pan. A seal of paraffin and beeswax is made between the periphery of the pan and its cover to preclude dirt.

TUBULATURES BETWEEN THE RESERVOIRS.—The tubulatures illustrated in figure 2 serve two functions: they act as channels for air and solution to move between the reservoirs, but they also determine and maintain the solution level in the constant-level reservoir.

Two parallel tubulatures having an inside diameter of not less than 5 mm. are required: the air channel with white rubber tubing (fig. 2 *A*) and the solution channel with red rubber tubing (fig. 2 *B*). Each is divided into a number of sections to increase ease of manipulation while also maintaining immovable the section determining the solution level. The sections, proceeding from the inverted reservoir to the constant-level reservoir, are as follows: The no. 6 rubber stopper closing the inverted carboy is fitted with two pieces of glass tubing, 7 mm. outside diameter, the one protruding into the neck of the bottle 2.5 cm. and the other, a part of the air channel, ending at the inner surface of the stopper. Both tubes extend below the stopper about 2 cm. Permanently attached to these are pieces of rubber tubing approximately 5 cm. long (fig. 2 *a*). The vertical arm of a glass elbow, 7 mm. in diameter and 3 cm. long on each arm, is inserted into the free ends of each of the rubber tubings (fig. 2 *b*). Attaching permanently to the horizontal arm of the elbow on each channel is a length of rubber tubing 2 or 3 cm. long (fig. 2 *c*), coupled to which by a 3-cm. piece of glass tubing is another 8–10 cm. length of rubber tubing (fig. 2 *d*). This latter tubing attaches permanently to the final section of the channels. This final section of the air channel (fig. 2 *e*), which must remain immovable, is of 7-mm. glass tubing (outside diameter) and protrudes 3 cm. into the reservoir. The end of it must be ground at an angle to prevent solution from hanging in it when air should enter.[3] The tubing terminating the solution channel enters through a no. 3 rubber stopper and protrudes approximately 5 cm. into the reservoir (fig. 2 *f*). It is immovably fixed, and the end of it need not be ground. The tubulatures from the left and right inverted reservoirs are the same.

[3] A suitable grinding medium is no. 70 carborundum lubricated at the time of using with turpentine or glycerin.

AIR-TUBULATURE ADJUSTMENT.—A screw adjustment is fixed for slightly raising or lowering the air intake, which in turn increases or decreases the level of solution in the constant-level reservoir as may be required for bringing several units of the apparatus to the same rate of flow. The same adjustment is used to prevent the two carboys from emptying simultaneously. It is composed of a machine screw and pivoting clamp, as shown in figure 2 *C*.

CAPILLARY SIPHONS.—Heavy walled (5 mm. outside diameter) capillary glass tubing having an inside diameter of 1 mm. is desirable for use as siphons to carry solution from the constant-level reservoir to the plant containers. They may be of any length or shape for special purposes. For general use a siphon 8 inches long, bent as illustrated in figure 2 *E*, has served best. The arm extending into the reservoir is $3\frac{1}{2}$ inches long; the free arm is 3 inches long. Siphons of larger bore make the precise adjustment of rate of flow more difficult. The drip from each siphon is caught in a glass funnel (fig. 1 *G*) through which it runs by a larger bore channel (5 mm. inside diameter) to the plant container.

CAPILLARY-SIPHON ADJUSTMENT.—The rate of flow of solution to the plant containers is regulated by raising or lowering the outlet of the capillary siphons. The mechanism for adjusting the inclination of these siphons consists of two units, one for rough adjustment (fig. 3) and the other for

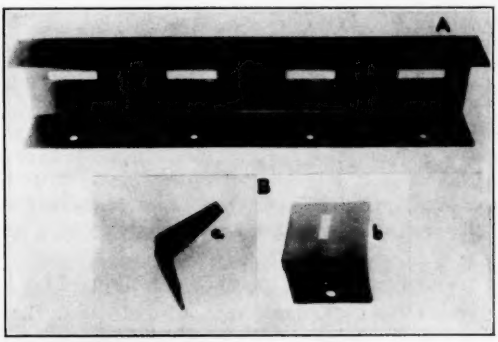

FIG. 3. Capillary-siphon rough adjustment: *A*, *B*, the two primary parts; *a*, side view of part B; *b*, back view of part B.

fine adjustment (fig. 2 *F*).

The rough adjustment consists of two parts as illustrated: (1) a length of heavy sheet metal bent as shown in figure 3 *A*, 14 inches long, $2\frac{1}{2}$ inches high, and $1\frac{3}{4}$ inches wide. Into it four horizontal slots $\frac{5}{16}$ inch $\times 1\frac{1}{2}$ inch are cut (if more than four capillary siphons are to be used, a proportional increase in the length of this piece and the number of slots should be made);

(2) a piece of right-angled iron or heavy sheet metal $2\frac{1}{2}$ inches wide (fig. 3 *B*). The vertical and horizontal sides are also approximately $2\frac{1}{2}$ inches. In the vertical face a $\frac{5}{16} \times 1\frac{1}{2}$ inch slot is cut. This face attaches by means of a machine screw to the metal piece just described. Thus both vertical and horizontal movement are possible. When the desired position is attained the screw is tightened and the adjustment remains permanently. In the horizontal face of the right angle piece is a $\frac{5}{16}$ inch hole through which the fine adjustment attaches by means of a machine screw, provided with a winged nut. All screws and nuts are galvanized to prevent rusting. This metal piece may be dispensed with for certain purposes, the angle-iron attaching them to the table directly.

TRAINS OF INVERTED RESERVOIRS.—Trains of carboys may be set up for cultures in remote places where more frequent renewal of reserves is un-

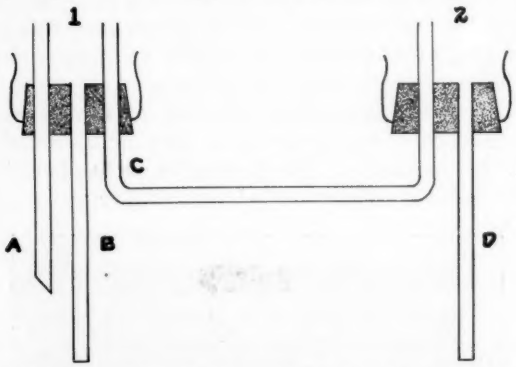

FIG. 4. Tubulature arrangement for connecting inverted carboys in trains: *A*, air channel from inverted reservoir 1 to constant-level reservoir; *B*, *D*, solution channels from inverted reservoirs 1 and 2 respectively to same constant-level reservoir; *C*, air channel connecting inverted reservoirs 1 and 2.

desirable. The connecting tubulatures shown in figure 4 make possible such an increase in the reserve nutriments without disturbing the advantages of the mechanism already described. The same type of connection may be made between the left and right inverted reservoirs as an alternative method to that just described.

CONTROL OF ALGAE IN SAND CULTURES.—For ordinary purposes the nutrient solution is led into the sand through a blackened glass tube. Clean white quartzite pebbles having a diameter of about $\frac{1}{4}$ inch are spread 2 inches deep over the top of the sand, preventing the growth of algae on the sand surface while at the same time cutting down evaporation.

Precision of apparatus

Table I gives the rates of flow from the capillary siphons of two 4-siphon units for consecutive 24-hour periods, when the rate of flow from each siphon was arbitrarily set at 14 drops per 2 minutes. By manipulating the

TABLE I

Record of drip rate for consecutive 24-hour periods, from two units, each siphon of which is set to deliver 14 drops each 2 minutes*

Day	Unit A				Unit B			
	1	2	3	4	1	2	3	4
	Right side, carboys closed							
1	800	810	840	810	880	890	870	790
2	860	855	885	860	860	870	850	820
3	935	930	935	950	925	935	910	870
	Left side, carboys closed							
4	960	930	930	940	960	930	940	950†
5	969	932	932	960	978	988	969	969
6	969	951	924	969	1006	1015	997	1006
Average delivery	915.5	901.3	907.7	914.8	934.8	938.0	922.7	900.8

* As the carboys empty, increasing amounts of solution are delivered, but all units vary in the same direction.

† Adjustment altered.

adjustments slightly, the drips may be brought into even closer agreement.

As noted in table I, the rate of nutrient delivery increases as the carboys empty, and as the flow changes from the left to the right carboy, but under similar conditions all units vary in the same direction and to the same degree; hence this variation should not enter as a disturbing element in large-scale experiments calling for several units.

Changes of temperature cause fluctuations in flow, as shown in table II, but under the usual range of conditions their magnitude is slight.

Table and plant containers

A table to support the mechanism has been designed for use with pineapple plants (fig. 1). Several units may be combined as a single table to replace the standard planthouse bench. Modifications may be made to adapt this apparatus for use with revolving tables or constant temperature devices.

The position of the siphons is the important factor in controlling the rate of flow of the nutrient solution. The shield (fig. 5) not only protects the siphons from movement or breakage by the plants, but covers the fun-

TABLE II

INFLUENCE OF TEMPERATURE ON RATE OF NUTRIENT SOLUTION DELIVERY SHOWN BY QUANTITIES OF LIQUID DELIVERED IN 2-HOUR PERIODS BY CAPILLARY SIPHONS OF SINGLE DRIP UNIT

TIME	AVERAGE TEMPERATURE*	QUANTITY OF FLOW			
		DRIP 1	DRIP 2	DRIP 3	DRIP 4
	° C.	cc.	cc.	cc.	cc.
8–10 A. M.	27.8	70.0	71.0	70.5	71.5
10–12	30.0	74.0	75.5	76.0	77.0
12– 2 P. M.	30.7	70.0	72.0	72.5	72.5
2– 4	29.3	77.5	80.0	79.0	79.5
4– 6	26.3	71.0	73.0	73.0	72.0
6– 8	24.8	67.5	69.0	68.5	68.0
8–10	23.7	65.5	67.0	66.5	66.0
10–12	23.2	64.0	65.0	65.0	65.0
12– 2 A. M.	22.7	61.0	62.0	62.0	62.0
2– 4	22.7	63.0	64.0	64.0	64.0
4– 6	23.3	64.0	65.5	65.0	65.0
6– 8	24.5	65.0	66.5	66.0	66.0
8–10	27.0	70.0	71.5	71.0	71.0
10–12	28.5	73.0	75.0	74.5	74.5

* Computed thermograph record readings made each 30 minutes.

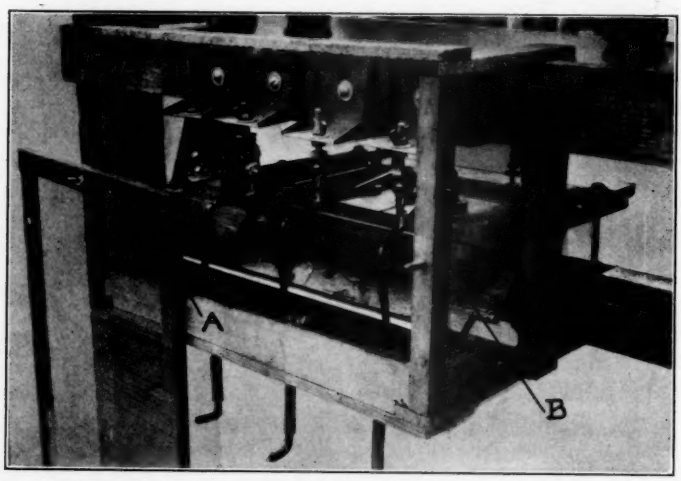

FIG. 5. Siphon shield for protecting capillary siphons from breakage. The funnels serving as semi-flexible couplings in solution channels are inclosed in the black compartment in lower, forward portion of shield. Cover *A* to the shield and cover *B* to the funnel compartment were moved out of place before photographing.

nels as well, limiting evaporation and the growth of algae. The funnel cover is an opaque compartment painted black on the inside, situated in the lower forward part of the siphon shield as shown in figure 5. The capillary siphons enter the funnels through suitable openings in the top of the funnel cover. Glass wool is used to close the space remaining between each siphon and its opening. The funnels (figs. 1 *G*, 5) serve as a coupling in the solution channel from the capillary siphons to the plant containers, allowing enough movement of the channel to prevent breakage.

Where danger of breakage is not a factor, the shield may be omitted and the siphons increased in length to connect directly with the plant container.

Summary

1. With care, an adjustment of siphons can be made so that variation in amount of solution delivered by a series of units does not exceed 50 cc. per liter; that is, 5 per cent. In view of the low concentrations of nutrient solutions this amount is so small a variation in the actual amount of any one element or compound delivered as to seem inconsequential.

2. More than one rate of drip may be served from a single constant-level reservoir, since rate of flow is determined by the inclination of individual capillary siphons.

3. Rate of flow of nutrients may be altered according to the requirements of the growing plant, without seriously interfering with the study in progress. The siphon easily can be adjusted to deliver more or less nutrient, and the new rate of flow can be measured for a short interval without disturbing the cultured plants.

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UNIVERSITY OF HAWAII

GROWTH MODIFICATIONS IN CITRUS SEEDLINGS GROWN FROM X-RAYED SEED

C. P. HASKINS AND C. N. MOORE

(WITH SEVEN FIGURES)

A summary is made in the present paper of certain morphological variations observed in seedlings of the citrus fruits arising from seed exposed to x-rays before planting. The work has been undertaken in the course of a series of investigations on the biological effects of x-rays and cathode rays, and although as yet no cytological examination of the material has been made, it was considered worth while to assemble descriptions of certain morphological effects at the present time, in view of the paucity of x-ray work reported with the citrus fruits, especially at higher voltages and currents. Certain of the variants considered have already been described,[1] but have been included here in order to complete the series.

Seeds of lemon, lime, tangerine, grapefruit, and sweet and sour orange were obtained through the kindness of Dr. R. G. LARUE, Superintendent of Cultivation of the University of California Agricultural Experiment Station, and Dr. A. F. CAMP, Head of the Department of Horticulture of the University of Florida. They were dried previous to shipment. A portion of the seed was x-rayed in the condition in which it was received. The remainder, before treatment, was soaked in distilled water for 15 minutes, then left in a completely moisture-saturated atmosphere for 12 hours. The seeds were then dried on filter paper and exposed at once. Treated seeds were planted immediately in seed flats, a mixture of one-third peat moss and two-thirds sharp sand being used, the seeds being covered in most cases with pure sharp sand. The seeds were germinated in an electrically heated greenhouse, day temperatures being maintained constant at 75° F. and night temperatures at 55° F. during the early growth of the plants. At a later time a large number of apparently normal seedlings were transferred to the open nursery, being planted in a sandy loam soil. Most of the more conspicuous variants were maintained in the greenhouse for a full year, however, being transferred to benches containing a mixture of sand, clay, and manure at the age of a few months. After this change they were not disturbed until the close of the experiment.

A Coolidge water-cooled tungsten-target tube of the thick-walled type was used as the source of x-rays, operated at 200 k.v.p. and 30 ma. from a

[1] Science, Aug. 5, 1932.
Science, Feb. 15, 1933.
Bot. Gaz. 94: 801–807. 1933.

high-tension transformer equipped with Snook mechanical rectifier. The seeds were exposed on a lead-covered table at a focal distance of 50 cm. Under these conditions the incident energy input, as measured with a graphite-covered collodion wall, radium-compensated Failla ionization chamber, was 165 roentgens per minute. The shortest wave length theoretically obtainable at this voltage is 0.062 Å. as calculated from the quan-

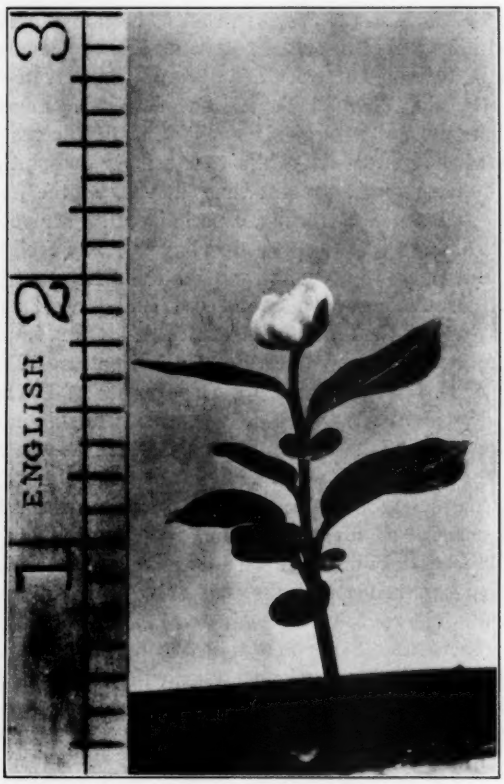

FIG. 1. Grapefruit seedling flowering prematurely after x-ray treatment of seed from which it came. Dosage 300 r.

tum relation $Ve = hv_{max}$. No metal filter was used, but the glass of the wall interposed a filtering action equivalent to about 0.10 mm. of copper. At 0.70 Å. the intensity of the emergent radiation was only about 0.3 per cent. of that impinging on the inner wall, as calculated from COMPTON'S 1926 value for the mass absorption coefficient of copper,[2] and this may be con-

[2] COMPTON, A. H. X-rays and electrons.

sidered the cutoff point. Substantially all of the radiation incident was included between these points, the greatest intensity lying within the $K_a - K_b$ doublet region for tungsten, between 0.21 and 0.18Å.

Several very curious effects were observed among the seedlings, which may be summarized here.

Premature flowering

Premature flowering is not infrequently observed in citrus seed beds, particularly in cases in which the acidity of the soil has not been properly

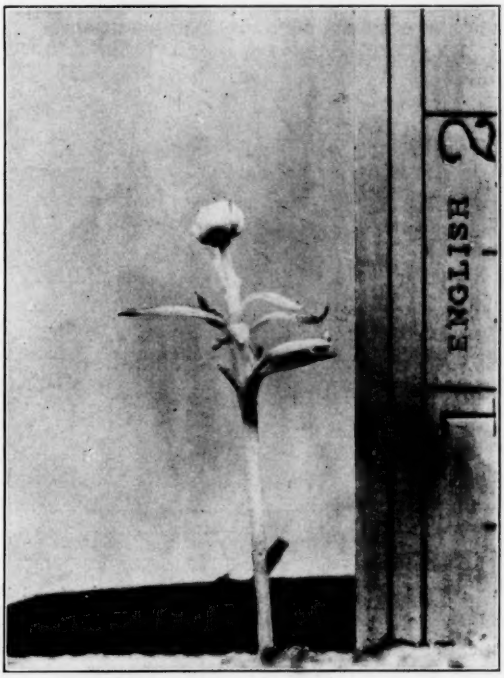

FIG. 2. Premature flowering of a chlorophyll deficient grapefruit seedling. Dosage 1300 r. in seed stage.

regulated. In the present case, however, the habit was pushed to an extreme which proved very interesting, especially in view of the fact that the hydrogen ion concentration of the seed beds was maintained within limits which, in the opinion of the citrus growers, should discourage early flowering.

Among one group of grapefruit seedlings arising from seeds which had been given x-ray dosages of from 300 to 1300 roentgens in both the wet and

dry states under the conditions described, two came into flower within six weeks of the time of sprouting. The flowers matured rather slowly, but, although diminutive, they proved to be normal in form. The plants, as shown, still carried the first pair of leaves intact, as is usual with citrus seedlings of this age. The plant shown in figure 1 was normal in leaf and flower coloration, and the leaves were nearly normal in form although somewhat elongate and diminutive. The other seedling (fig. 2) was extremely deficient in chlorophyll and the flower was imperfectly pigmented, being of a yellowish white color similar to the leaves. The stamens, however, were golden. The green plant received a dosage of 300 roentgens and the white one a dosage of 1300 under the same conditions.

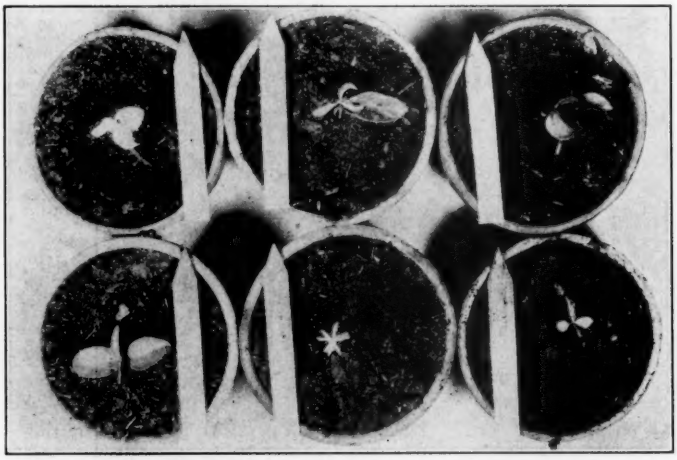

FIG. 3. Albinism of citrus seedlings following x-ray treatment in the seed stage.

The plant deficient in chlorophyll died very shortly, as was to be expected. The normally pigmented seedling, although deficient in roots, survived and grew slowly, and, when one year of age, again showed a flower bud on new growth, although it did not come to maturity.

At the age of nine months, 10 per cent. of this entire planting came into blossom almost simultaneously, the flowers in this case being of normal petal number and almost of normal size. Some of the flowering seedlings were of normal stature for their age, while others were distinctly dwarfed.

Albinism

Albinism, like premature flowering, is a character not infrequently apparent among citrus seedlings, especially in highly hybridized strains. In

the present case, however, an abnormally high percentage of seedlings showed this deficiency. It ranged from a condition best described as virescent, in which by no means all green pigment was lacking, through partial deficiencies in which a deep green was mottled irregularly throughout the leaf on a white background (fig. 3), to the complete whiteness characterizing one of the flowering types mentioned in the last section, and very many plants otherwise normal in appearance. Although albino plants were not absent from control plantings (as they very rarely are), the percentage of deficiencies in treated groups was manyfold higher.

Fasciation

Numerous cases of terminal and lateral bud fasciation were observed, the most pronounced of which is illustrated in figure 4, right. This speci-

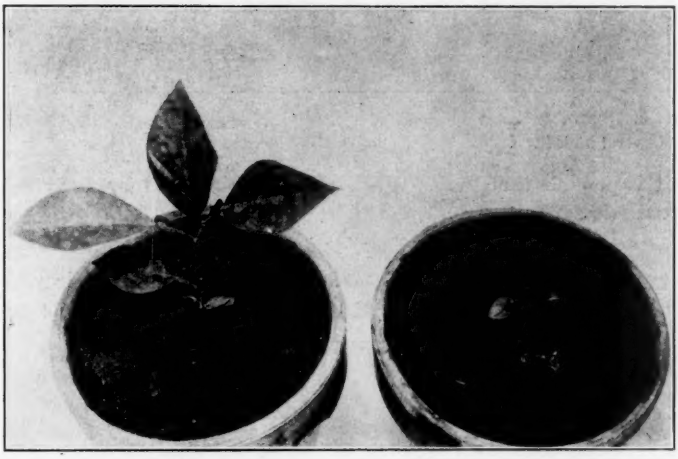

FIG. 4. Bud fasciation in *Citrus aurantium* following x-ray treatment of seeds. Control at left.

men of *Citrus aurantium,* when photographed, was but little over an inch in height. Control specimens, as is usual with this species, grew vigorously, and had attained a height of 10 inches when the photograph was taken. Clearly the primordia only of the later true leaves were affected, since the plant appeared normal in the first few weeks of life. Curiously the later leaves, although diminutive, were of nearly normal form.

Twisting

Two young seedlings from the time of sprouting showed a decided tendency to spiral in a counter-clockwise direction. Both plants twisted so markedly as to bend the stem from the vertical and to crush the leaves

against the stem (fig. 5, extreme right). After six months the habit was abandoned and later growth was normal. Both plants showed some evidence of tissue inversion and other characteristic x-ray injury during early

FIG. 5. Spiral twisting of stems of citrus seedlings following x-ray treatment of seeds. Control at left.

life. It is possible that the condition was brought about by x-ray induced abnormal mitoses.

Duplication

Very many instances were noted in which the midribs of leaves were split near the base, both halves being nearly equally robust. In a number

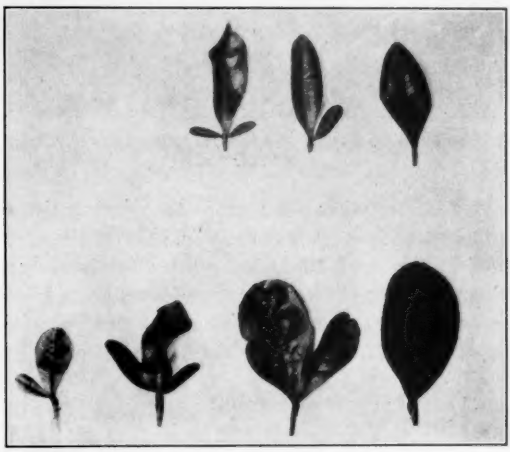

FIG. 6. Leaf modifications in citrus seedlings following x-ray treatment in the seed stage. See also fig. 5, center.

of cases, especially with the grapefruit, the entire leaflet was duplicated, the resulting two terminals usually being of nearly the same dimensions (fig. 5, center, and fig. 7). In lemon stock similar developments gave rise to partially bifoliate, bifoliate, partially trifoliate, and truly trifoliate leaves, often coexisting on the same plant (fig. 6).

FIG. 7. Grapefruit seedling with modified leaf, and accessory cup-like leaf. X-ray treatment given in seed stage.

Peloric leaf formation

An interesting development of a cup-shaped type of peloric leaf occurred in two grapefruit seedlings from x-rayed seeds, in each case the leaves proceeding laterally from the stem at a point some distance below the terminal bud. The formation was new in the experience of the writers and in that of a citrus horticulturist who examined it. The peloric leaves persisted for more than a year, the plants bearing them being otherwise normal.

Work is being continued in the field, and through the kindness of Mr. W. J. PLATTEN, of Sebastian, Florida, grafts of several of these variants have been made on to rough lemon stock, with a view to checking any possible modifications in fruit.

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CAROTENE AND XANTHOPHYLL IN PINEAPPLES[1]

O. C. MAGISTAD

The color of the flesh of the fruit in pineapples varies from white or colorless to a deep yellow. The commercial varieties in the Territory of Hawaii, the Cayenne and Hilo, are both intermediate in shade. Within these varieties depth of color varies greatly from place to place, and even from fruit to fruit within a single field. The present investigation was undertaken to determine the nature of the pigments in the fruit of pineapples and to measure the quantity of pigment present.

Experimentation

Using the chemical methods of separation based on solubilities, as given by WILLSTÄTTER and STOLL (**10**), SCHERTZ (**7, 8, 9**), and PALMER (**6**), it was found that carotenes and xanthophyll were present in pineapple flesh together with anthocyanins, flavones, and traces of chlorophyll. The yellow colored shell of pineapple fruits was found to be relatively rich in carotenes and flavones, while the green leaves contained in addition to chlorophyll even larger concentrations of flavones, carotenes, and xanthophylls than were found in the fruit flesh.

METHODS.—The finely chopped fresh material was extracted with acetone and the pigments present were transferred and concentrated in ether. Flavones were removed by washing with a 1 per cent. aqueous solution of sodium carbonate. Chlorophyll was saponified and separated from the carotenes and xanthophylls. The ether was evaporated off at temperatures below 40° C., and the carotenes and xanthophylls were separated in the usual manner on the basis of solubilities in methyl alcohol and petroleum ether.

Small violet red crystals formed on pouring a petroleum ether solution of carotene into methyl alcohol. Crystals also formed on evaporation of carbon bisulphide solutions of carotene. These appeared under the microscope to be identical with those described by WILLSTÄTTER and STOLL (**10**, plate III). Carotene isolated from pineapple tissue gave a blue coloration with the antimony trichloride reagent of CARR and PRICE (**2, 3**).

The amount of chlorophyll present was estimated by comparison against a synthetic solution as advocated by GUTHRIE (**4**).

The quantity of carotene present was determined by colorimetric comparison with standard yellow Lovibond slides as described by SCHERTZ (**7**). When compared with solutions of potassium bichromate as advocated by

[1] Published with the approval of the Director as Technical Paper no. 73 of the Pineapple Experiment Station, University of Hawaii. This paper was presented at the Boston meeting of the American Society of Plant Physiologists, December 28, 1933.

PALMER (**6**), fair agreement was obtained. A weighed quantity of pure carotene obtained from the Smaco Corporation when dissolved in petroleum ether gave readings checking well with the curves of SCHERTZ and of PALMER, and with the solution of carotene isolated from pineapples.

Xanthophylls were determined by colorimetric comparison against standard yellow Lovibond slides as advocated by SCHERTZ (**8**).

AMOUNTS OF PIGMENTS PRESENT

A medium sized pineapple plant with a ripe fruit was analyzed for the pigments chlorophyll, carotene, and xanthophyll. The results obtained are shown in table I.

TABLE I

PIGMENT CONTENT OF A PINEAPPLE PLANT

PLANT PART	FRESH WEIGHT	CHLOROPHYLL		CAROTENE		XANTHOPHYLL	
		PER 100 GM.	TOTAL	PER 100 GM.	TOTAL	PER 100 GM.	TOTAL
	gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Lower leaves	1056	65.50	691.68	3.75	39.60	2.05	21.65
Middle leaves	1598	50.65	809.39	1.85	29.56	1.43	22.85
Upper leaves	1012	48.00	485.76	2.38	24.09	0.71	7.19
Shoots	1162	36.25	421.23	2.38	27.66	1.17	13.60
Crown	145	26.58	38.54	1.05	1.52	1.20	1.74
Fruit stalk	151	3.95	5.96	0.28	0.42	0.27	0.41
Stem	1215	1.45	17.62	0.06	0.73	0.06	0.73
Flesh	1786	Trace		0.15	2.68	0.03	0.54
Core	214	"		0.20	0.43	0.04	0.08
Shell	930	"		0.33	3.07	0.33	3.07
Total	9269		2470.18		129.76		71.86

A study of this table shows first that the chlorophyll content of the relatively thick leaves of pineapple plants is lower than for thin leaves of common trees and plants. Thus, WILLSTÄTTER and STOLL (**10**, page 102) give values up to 300 mg. chlorophyll per 100 gm. green tissue for some plants. Again most plants contain more xanthophyll than carotene in their leaves, but in table I the pineapple plant is shown to have more carotene than xanthophyll throughout all its parts. The absolute quantity of carotene and xanthophyll present is small in comparison with most plants (**10**, page 102). Of special interest is the relatively low content of xanthophyll in the fruit flesh.

CAROTENOID PIGMENT CONTENT OF A NUMBER OF VARIETIES AND HYBRIDS OF PINEAPPLE FRUITS

A study was made to determine the quantity of carotene and xanthophyll present in the fruit flesh of various pineapple varieties. The fruits

were all grown in the Territory, those other than Cayenne and Hilo being grown by the Genetics Department at the substation at Wahiawa. The results are listed in table II.

TABLE II

CAROTENE AND XANTHOPHYLL CONTENTS OF FRUIT FLESH OF DIFFERENT PINEAPPLE VARIETIES

VARIETY	NUMBER OF FRUITS	PER 100 GM. FLESH		BOTH	RATIO C/X
		CAROTENE	XANTHOPHYLL		
		mg.	*mg.*		
Cayenne					
Lot 1	13			0.13 ± 0.004	
Lot 2	9	0.22 ± 0.011	0.026 ± 0.005	0.25 ± 0.012	8.5
Lot 3	2	0.15	0.011	0.16	13.6
Lot 4	12	0.18 ± 0.014	0.015 ± 0.002	0.20 ± 0.014	12.0
Hilo	24			0.15 ± 0.008	
7966	10			0.50 ± 0.03	
Natal	10			0.45 ± 0.03	
Ruby	10			0.41 ± 0.04	
Queen	5			0.38 ± 0.03	
7898	3			0.38	
8053	10			0.26 ± 0.02	
4552	11			0.17 ± 0.012	
8680	11			0.044 ± .0006	
Bermuda	10			0.031 ± 0.005	
8232	5			0.01	

The first part of the table gives the variation in pigment content of four lots of Cayenne. In these lots the carotene plus xanthophyll content varied from 0.13 to 0.25 mg. per 100 gm. of fruit flesh. Not infrequently fruits will contain even less than 0.13 mg. pigment per 100 gm. flesh. The data in the latter part of table II were obtained by estimating carotene and xanthophyll together, without separation.

A comparison of Cayenne with the other varieties indicates that some contain far more, and others much less pigment. Thus 7966, Natal, Ruby, Queen, and 7898 contain about twice as much pigment as does Smooth Cayenne; while some varieties and hybrids, such as 8680, Bermuda, and 8232 are almost devoid of pigment and appear very white-fleshed.

The range in carotenoid pigment content of pineapple fruits of various varieties is strikingly similar to the range observed by BILLS and MCDONALD in the carotene content of ten carrot varieties (1). They found Early Scarlet Horn to contain 9.6 mg. of carotene per 100 gm., while Isbell's Maude S on the other end of the series contained only 0.12 mg. carotene per 100 gm. Thus the most colorless carrot contains about as much pigment

as does Cayenne pineapple, while some carrot varieties contain about 40 to 60 times as much.

PERMANENCE OF CAROTENE AND XANTHOPHYLL IN CANNED PINEAPPLE

During 1933, three lots of pineapple packed as indicated in table III were opened and their pigment content determined. The results appear in the table.

TABLE III

CAROTENE AND XANTHOPHYLL CONTENT OF STORED CANNED PINEAPPLES

LOT	NUMBER OF SAMPLES	PER 100 GM. FLESH		RATIO C/X
		CAROTENE	XANTHOPHYLL	
		mg.	*mg.*	
1927 Pack, tins	10	0.088 ± 0.007	0.011 ± 0.001	8.4
1927 Pack, glass	10	0.121 ± 0.004	0.017 ± 0.002	7.1
1932 Pack, tins	18	0.097 ± 0.004	0.015 ± 0.006	6.5

No record is available of the pigment content of the pineapple fruits represented in table III at the time of canning. However, since the amounts present are about equal to the amount found in fresh pineapples, it can tentatively be concluded that there is no marked loss of carotene and xanthophyll during storage. The 1927 pack in tins and in glass was a homogeneous lot at the time of packing and should have had the same pigment content at that time. The greater quantity found in the glass packed material on examination in 1933 suggests that there is less decomposition of pigment when packed in glass as compared with tin.

Discussion

The yellow color of pineapple flesh is due to the presence of carotene and xanthophyll. These pigments are soluble in the fat solvents within the chromoplasts. Carotene predominates over xanthophyll. No attempt has been made to determine which carotenes were present nor the proportion of each.

A number of recent investigations have proved carotene to be a precursor of vitamin A. The β carotene is markedly superior to α and γ carotene. As a source of vitamin A, pineapples in this respect (**2, 5**) rank far behind such vegetables as lettuce, spinach, and carrots.

Summary

Pineapple fruits owe their yellow color to carotene and xanthophyll, carotene predominating. The quantity of carotene present ranges from about 0.10 to 0.25 mg. per 100 gm. flesh.

The writer wishes to express appreciation to Dr. M. B. LINFORD for aid in this study, and to Dr. J. L. COLLINS for samples of the hybrids.

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BRIEF PAPERS

LIGHT AS A FACTOR INFLUENCING THE DORMANCY OF LETTUCE SEEDS[1]

Lettuce seeds of the highest vitality may fail to germinate when tested in a closed chamber germinator. Seed analysts have observed that germination may be induced in many of these dormant lettuce seeds by presoaking them in cold water for 2 hours. However, such increase in germination is due not to the soaking in water but to the action of light on the wet seeds.[2]

Recent investigations by the writer show that it is even unnecessary to soak the seeds in cold water since the dormant condition can be broken merely by placing the seeds in an atmosphere saturated with water vapor and giving a longer exposure to light. Lettuce seeds of the Grand Rapids variety which were placed in a humid atmosphere and exposed to light for 10 hours and then placed on moist blotters at 20° C. in the dark germinated 98 per cent. A duplicate test which had received the same treatment but which was not exposed to light germinated only 8 per cent. The period of exposure to light can be shortened to 2 minutes or perhaps less by allowing the seeds to take up moisture from the air before exposing them to light. This germination response is of importance because it shows that water in the form of a film surrounding the seed is not essential to the breaking of the dormant condition by light.

Whether or not lettuce seeds in which the dormant condition has been broken by exposure to light will remain non-dormant when dried depends upon the physiological condition of the seed, the period of exposure to light, the temperature, and the manner in which the drying process is conducted. Lettuce seeds of the Big Boston variety which were placed in a humid atmosphere and exposed to light for a period of 24 hours at 23° C. and, without drying, placed on moist blotters at 20° C. in the dark, germinated 98 per cent. A similar lot of seeds which had received the same treatment but which was dried in the dark germinated 35 per cent. The failure of lettuce seeds to retain the non-dormant state when dried appears to be a characteristic response of lettuce seeds which require a temperature of 22° C. or lower for germination. It has been determined that if the seeds are set over water, exposed to light for 6 hours, and then placed in a refrigerator at a temperature of 6° C. for 10 days, they can then be dried and still retain the non-dormant state. Furthermore, it has been observed that

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 47.

[2] SHUCK, A. L. Some factors influencing the germination of lettuce seed in seed laboratory practice. New York State Agr. Exp. Sta. Tech. Bull. no. 222. 1934.

exposure to light at the time of drying may have a marked influence on the percentage of dormant seeds. Moist Grand Rapids lettuce seeds which were exposed to light for 24 hours and then dried at room temperature in the dark germinated 92 per cent. at 20° C. A similar lot of seeds which were dried at the same temperature but in the light germinated only 5 per cent. The degree of dormancy originally existing in the seed appears to be the most important factor determining the necessary length of exposure to light and the germination response of the seed when dried.

The shorter wave lengths of the visible spectrum are more effective than daylight in producing a dormant condition in both presoaked and humidified lettuce seeds when they are again dried in the presence of light. Grand Rapids lettuce seeds were placed over water, exposed to light for 24 hours, and then dried in the light, in the dark, and under plates of spectral glass. The seeds were dried for 24 hours and then placed to germinate on moist blotters at 20° C. in the dark (table I).

TABLE I

INFLUENCE OF LIGHT AND DRYING ON THE RETENTION OF THE NON-DORMANT STATE IN GRAND RAPIDS LETTUCE SEEDS

TEST NO.	CHECKS, GERMINATION IN DARKNESS	PERCENTAGE OF SEEDS GERMINATING AT 20° C. IN DARK WHEN DRIED UNDER THE FOLLOWING LIGHT RELATIONS				
		IN DAYLIGHT	UNDER SPECTRAL GLASS			IN DARKNESS
			YELLOW	GREEN	BLUE	
	%	%	%	%	%	%
1	15	32	21	75	16	89
2	6	15	24	59	17	64
3	11	7	17	67	8	90
4	13	5	10	79	12	92
5	15	30	34	67	17	95
6	30	18	16	79	15	98
ave.	15	18	20	71	14	88

In one of the representative tests the seeds dried in the dark germinated 89 per cent., while those dried in the light germinated 32 per cent. Similar lots which were dried while exposed to the individual colors of the spectrum germinated as follows: violet 12 per cent., indigo 5 per cent., blue 16 per cent., green 75 per cent., yellow 21 per cent., orange 24 per cent., and red 15 per cent.

If the dormant state has been broken to such an extent that the seeds remain non-dormant when dried in the dark, then only a small percentage

of the seeds revert into dormancy when dried while exposed to the green rays, whereas if they are dried while exposed to either the red or the blue rays, a high percentage of the seeds become dormant. The violet, indigo, and blue rays are more effective in producing dormancy than the longer red rays since a semi-stable condition can frequently be produced in non-light-sensitive seeds by drying the moist seeds while exposed to the shorter wave lengths of light. The main factor influencing the germination response of seeds when dried while exposed to the different colors of the spectrum appears to be the degree to which dormancy has been broken, which state in turn is influenced by the temperature and the period of exposure to light. In order to duplicate these results it is suggested that the moist lettuce seeds be given an exposure to light for a period of 24 to 48 hours, since an exposure for only a few hours may not be sufficient to break the stable condition in the embryo while a prolonged storage over water will cause an increase of dormancy and finally deterioration of the seeds.

The influence of light on the germination of lettuce seeds has been studied by FLINT,[3] who has found that the longer wave lengths of the visible spectrum promote germination while the shorter wave lengths are not only ineffective in promoting germination but may inhibit it. Since the red rays are effective in breaking the dormant condition and promoting germination while the blue rays cause the formation of a semi-stable condition, it appears that there may be an intermediate region of the spectrum which has no appreciable influence on certain physiological processes in the embryo associated with the dormant state. That there is a region of the spectrum between the blue and the red rays which has less influence on the embryo than the rays on either side is suggested by the germination response of lettuce seeds after they are dried while exposed to the green rays. These observations have been made upon Grand Rapids lettuce seeds which usually exist in a dormant condition which makes them particularly sensitive to light. A corresponding semi-stable condition may or may not be present to such an extent in other varieties of lettuce seeds, and this fact must be taken into consideration when studying the response of different lots of seeds to light.

Lettuce seeds are continually changing organisms which show different degrees of dormancy depending on the age or physiological condition of the seeds. The seeds often exist in a dormant condition in which certain of the life processes necessary for germination appear to be in a state of delicate equilibrium. This condition can be broken by exposing the moist seeds to light. A similar unstable condition can be formed again by drying the seeds in daylight or by exposing them to the shorter wave lengths of the

[3] FLINT, L. H. Light in relation to dormancy and germination in lettuce seed. Science 80: 38–40. 1934.

spectrum. These transformations can be made in the light and without the presence of water in the form of a film surrounding the seed. It appears that under these conditions light does not facilitate the passage of any substance from the seed but causes certain unknown photochemical changes within the seed.—A. L. SHUCK, *New York State Agricultural Experiment Station.*

A CASE OF CHANGE OF TROPIC RESPONSE

(WITH ONE FIGURE)

During a recent trip into the northern woods of Minnesota the peculiar formation seen in the accompanying illustration (fig. 1) was encountered. This specimen of the black spruce was found near the shore of Lake Hole-in-the-wall, a small lake which is located about six or seven miles southeast of Marcel. The two lateral branches had each made a three-quarter turn and then grew almost directly upward. The upper portions of these two branches if seen alone would appear much like the top of the main axis of the tree, with laterals extending outward and somewhat drooping.

Since the lateral branches of the black spruce normally grow nearly at right angles to the vertical main axis and perpendicular to the force of gravity, they may be said to exhibit plagiotropic response. No attempt, however, was made to determine the specific influence or influences responsible for this position. After making the turn the branches apparently became definitely negatively geotropic like the main trunk.

The writer is not certain as to the cause of this abnormal formation but thinks that it might have been caused by the mistletoe, *Arceuthobium pusillum,* which is known to attack the black spruce in Minnesota and produces abnormal development. At the time of the visit to this region he was unaware of the occurrence of this mistletoe and failed to look for its presence on this individual tree which was the only one seen which seemed to be abnormal. These two branches, however, are thickened at the curvature and resemble mistletoe injury as reported by colleagues who are familiar with its pathogenic symptoms.

Aside from the peculiar unique formation, is the fact that these branches are seemingly unaffected in their subsequent growth and have later responded to growth forces in such a manner as to resemble the main axis of the tree, illustrating quite definitely a change of tropic response. Just what forces were brought into play to cause the branches to grow in such a way as to form the circle it is difficult to say. The parasite must have stimulated growth on the upper and outer sides of the curve and then, after the growing tip had passed the half circle mark, left the elongating portion unaffected. After that, the end of the branch completed the turn until it

FIG. 1. Abnormal branches of the black spruce (*Picea mariana*) which have changed their direction of growth to resemble the vertical main trunk of the tree.

became upright and thereafter continued to grow vertically. One would think that the branch, once it had reached the half circle mark, would have continued growing horizontally, for at this point it seems no longer affected by the parasite. This horizontal position would have been its normal orientation. Why it responded to gravity or other forces in a manner different from that of normal branches after making the half turn is problematical, but it seems quite certain that there had occurred a change of tropic response after the elongating tip portion of the branch had passed the half turn resulting in the later vertical growth.—W. J. HIMMEL, *University of Nebraska.*

NOTES

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists was held at Pittsburgh, December 27–29, 1934, in conjunction with the meetings of the A. A. A. S. The scientific programs were held in the Cathedral of Learning, University of Pittsburgh. There were four sessions for reading of papers, and joint meetings with the Horticulturists, Section G, A. A. A. S., and a joint symposium on plant hormones with the Physiological Section of the Botanical Society of America.

Business meetings were held by the Executive Committee on call by the President, and matters already in shape for action were attended to in the regular business meetings. The reports showed that the last year has been one of real progress. Membership is again increasing, and the finances are in sound condition.

The annual dinner was held at the University Club on December 27, with more than 100 in attendance. The dinner was made the occasion for election of corresponding members, announcement of awards, and a memorial address on Dr. KARL RITTER VON GOEBEL which was prepared by Prof. FRANCIS E. LLOYD, McGill University. In the enforced absence of the author of the memorial address, it was read by Dr. F. M. ANDREWS, Indiana University. This address will be published in PLANT PHYSIOLOGY in a later issue. At the close of the festivities, President LIVINGSTON was tendered a memorial of affection and appreciation by graduates of Johns Hopkins University in honor of his completion of 25 years of service to plant physiology at Johns Hopkins. Those who were present will treasure the memory of these unforgettable events.

Summer Meeting.—A summer meeting at Minneapolis and St. Paul late in June is in prospect. The arrangements will be made by the Minnesota Section, and members will be informed more fully in the April number of PLANT PHYSIOLOGY as to the exact time of the meeting, hotel accommodations, extent of the program, and social events if any. The summer meeting of the A. A. A. S. is scheduled for Minneapolis, and this circumstance provides an opportunity for a meeting with our oldest Section. Previous experience has demonstrated that our Minnesota members and friends are past masters in the arts of entertainment and organization of meetings. In extending a cordial invitation to all of the members of the Society to come to Minnesota, they hope that visitors may come not only from the middle west, but also from more distant regions.

Corresponding Members.—The second election of corresponding members of the American Society of Plant Physiologists occurred at Pittsburgh.

Two distinguished plant physiologists were honored: Dr. GOTTLIEB HABERLANDT of the University of Berlin, and Dr. V. N. LUBIMENKO of the Botanical Garden of the Russian Academy of Sciences at Leningrad. Both of these men have enjoyed long and active careers in plant physiology, and have made notable contributions to the advancement of our knowledge of plants and their behavior. The Society has honored itself in honoring them.

Life Membership.—The tenth award of the CHARLES REID BARNES life membership in the Society was made to Professor FREDERICK FROST BLACKMAN, Fellow of St. Johns College, University of Cambridge, and for many years Reader in Botany at Cambridge. He has been a Fellow of the Royal Society of London for nearly thirty years, and has been the guiding spirit of the physiological work at Cambridge for several decades. This is the first time that the award has been made to a scientist residing outside of North America. No happier choice could have been made at this time.

Stephen Hales Award.—The fourth award of the STEPHEN HALES prize was made to Dr. CHARLES A. SHULL of the University of Chicago. This prize was established at the Nashville meeting in 1927, and the previous prizemen are Dr. D. R. HOAGLAND, University of California; Dr. W. W. GARNER, U. S. Department of Agriculture; and Dr. H. B. VICKERY, Connecticut Agricultural Experiment Station. These men constituted the committee on the award for 1934. In the absence of the chairman of the committee, the citation was read by Dr. CHARLES F. HOTTES, University of Illinois, and the certificate of award was presented by the President, Dr. BURTON E. LIVINGSTON.

Program Committee.—The program committee for the St. Louis meeting in December, 1935, has been appointed. The personnel of the committee as announced by President LIVINGSTON is as follows: Dr. E. S. REYNOLDS, Missouri Botanic Garden, chairman; Dr. H. R. KRAYBILL, Purdue University; Dr. S. V. EATON, University of Chicago. The secretary-treasurer, Dr. A. E. MURNEEK, University of Missouri, is *ex officio* member of the committee.

Editorial Board.—At the end of our first decade, the constitution and by-laws of the Society are being carefully examined by the executive committee for possible improvements. Many changes are likely to be proposed. One of the most important changes will affect the size and constitution of the editorial committee for the official journal, PLANT PHYSIOLOGY. Provision will be made for appointment of some of the members of the editorial staff by the executive committee, and for election of some members by the

Society. The editorial staff will be enlarged possibly to eight members, five appointive and three elective for definite terms, so that more fields, such as horticulture, forestry, etc., can be represented on the board. Constitutional changes will wait on approval of the changes by ballot of the members; but by-law changes are put into effect on unanimous approval by the executive committee.

Cost of Reprints.—Arrangements are being made for a reduction in the price of reprints. In order to effect any saving to our members it will be necessary to have full cooperation of authors of papers. The prompt execution of orders at the time galley proofs are returned to the editorial office is a part of the plan. Blanks giving the prices charged according to length of the reprint will be attached to the galleys, and blanks are included for ordering reprints and for shipping directions. As in the past, 50 free reprints without covers will be added to the orders; but in case the author orders no reprints no gratis reprints are to be sent. Authors should keep these provisions in mind in making up orders.

Endowments.—Three of the endowment funds of the American Society of Plant Physiologists are in excellent condition. The general endowment is very much too small. The Society is in position to accept and administer gifts and bequests in accordance with the wishes of the donor upon the approval of acceptance of gifts for special purposes by the executive committee. An approved form for bequests will be sent to any one interested in making bequests to the Society. Cash gifts to the general endowment are welcome at any time, and will be put to work at once upon receipt. Occasional gifts have been made to the endowment during the last several years, and it is hoped that more friends may assist in the development of the Society in this practical way.

Portraits.—With the appearance of the April number of PLANT PHYSIOLOGY, two additional portraits will be added to the collection of prints available for distribution. The full set of 20 reprints of portraits may be had for $2.00 plus postage and insurance.

Water Relations Reprints Wanted.—A new edition of "The plant in relation to water" by N. A. MAXIMOW is in preparation. The author of this work would appreciate it very much if he might receive reprints of important contributions on this subject from all American investigators who have worked in this field. The international character of scientific knowledge makes such cooperation very desirable. Those who wish to assist him should address Professor N. A. MAXIMOW, Institute of Grain Farming, Postal Agency 20, Saratov, U.S.S.R.

Statistical Methods.—The fifth edition of R. A. FISHER's Statistical Methods for Research Workers has been published by Oliver and Boyd, Tweeddale Court, Edinburgh, or 33 Paternoster Row, London E.C. The changes are again insignificant. Section 5 is now a historical note; section 49.1 on analysis of covariance has been enlarged; section 21.01 considers YATES's correction for continuity; and 21.02 gives the exact treatment of 2×2 tables. It is a useful work for those who are obliged to master and use statistical methods in research. Most investigators would find it advisable to be familiar with the practical usefulness of statistics in testing their results. The price of the volume is the same as for the earlier editions, 15 shillings net.

Excretion and Secretion.—Volume 32 of the Monographien aus der Gesamtgebiet der Physiologie der Pflanzen und der Tiere is entitled Die Stoffausscheidung der höheren Pflanzen. The author is Dr. A. FREY-WYSSLING of the Federal Technical Highschool, Zürich. The introduction sets forth the scope of the work, and defines the various forms of elimination of materials from the plant body. Section I considers the deposition of cell walls, the substances laid down in them, and the structures that appear, such as the micellae, intermicellar spaces, and intermicellar deposits; the physiology of the cell walls is summarized in the latter part of this section. Section II deals with "recretion," the removal of inorganic elements that have been taken in from the soil, function for a time in the plant, and are then deposited in crystalline or other insoluble form. Calcium salts and silicon dioxide deposits are the two main "recretions." Section III deals with excretion proper, and section IV with secretion. A brief summary closes the text. There is a bibliography of about ten pages, a subject index, and 128 figures. It is a useful summary of the last stages of metabolism in plants. The book may be obtained from the publisher, Julius Springer, Berlin, at RM 28 for brochure copies, and RM 29.4 for cloth bound.